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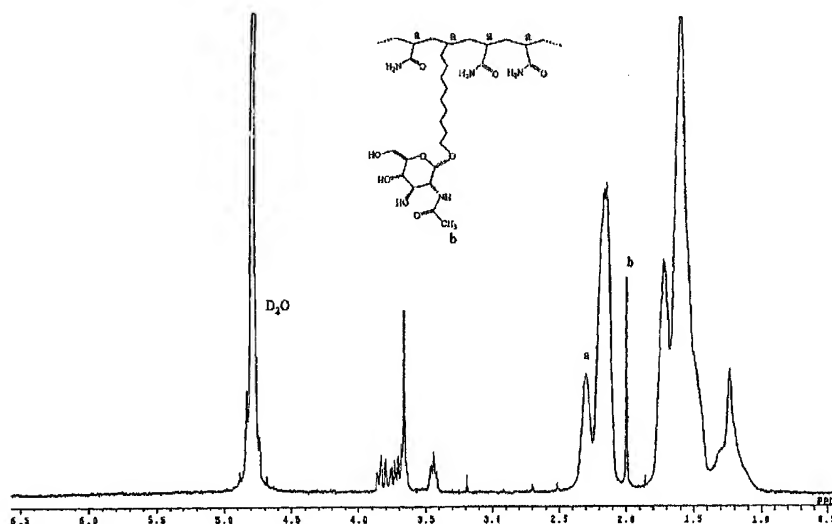
(43) International Publication Date
18 July 2002 (18.07.2002)

PCT

(10) International Publication Number
WO 02/055021 A2

- (51) International Patent Classification⁷: **A61K** F-94400 Vitry-sur-Seine (FR). **BASKARAN, Subramanian** [IN/US]; 70 Rock Harbor Lane, Foster City, CA 94404 (US).
- (21) International Application Number: PCT/US02/01030
- (22) International Filing Date: 14 January 2002 (14.01.2002) (74) Agents: **WINNER, Ellen, P.** et al.; Greenlee, Winner and Sullivan, P.C., Suite 201, 5370 Manhattan Circle, Boulder, CO 80303 (US).
- (25) Filing Language: English
- (26) Publication Language: English (81) Designated States (*national*): AU, CA, JP, US.
- (30) Priority Data: 60/261,275 12 January 2001 (12.01.2001) US (84) Designated States (*regional*): European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR).
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— without international search report and to be republished upon receipt of that report
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(54) Title: GLYCOPOLYMERS AND FREE RADICAL POLYMERIZATION METHODS



(57) Abstract: A glycopolymer composition is provided comprising glycopolymer molecules having a polymer backbone; a first pendent unit comprising a linking group connected to said polymer backbone and a saccharide moiety connected to said linking group, optionally a second pendent unit; a phenyl ring at a first end of the polymer backbone; and a cyanoxyl group at the second end of the polymer backbone, useful as intermediates for making bioactive glycopolymers which bind to bioactive molecules, viruses, cells and substrates for protein separation, cell culture, ad drug delivery systems, as well as in targeting for treatment of wound healing and other pathological conditions.

GLYCOPOLYMERS AND FREE RADICAL POLYMERIZATION METHODS

CROSS-REFERENCE TO RELATED APPLICATIONS

5 This invention claims priority to U.S. Serial No. 60/261,275 filed January 12, 2001, which is fully incorporated herein by reference to the extent not inconsistent herewith.

GOVERNMENT FUNDING

10 This invention was made with support by a grant from the National Institutes of Health (RO1HL60464). The government may have certain rights in this invention.

BACKGROUND

15 Glycosaminoglycans (GAGs) are naturally occurring linear polysaccharides encountered both in the extracellular matrix and on cell surfaces where they form a carbohydrate coating referred to as the glycocalyx. GAGs are involved in a wide array of physiological processes, including cell proliferation and migration, as well as modulation of angiogenesis and inflammatory responses (Clowes, A.W. and Karnovsky, M.S. (1977) *Nature* 265; Jackson, R.L. et al. (1991) *Physiol. Rev.* 71:481; Linhardt, R.J. et al. (1996) In
20 Biomedical Functions and Biotechnology of Natural and Artificial Polymers, ATL Press, p. 45). The diverse bioactivities of GAGs are a consequence of unique binding sequences that facilitate local sequestration of biologically active proteins, such as growth factors and antithrombin III. In this manner, GAGs function as delivery vehicles for the controlled local release of a variety of proteins and, in select circumstances, potentiate the activity of
25 the bound protein (Kjellen, L. and Lindahl, U. (1991) *Ann. Rev. Biochem.* 60:443; Faham, S. et al. (1996) *Science* 271:1116; Bitomsky, W. and Wade, R.C. (1999) *J. Am. Chem. Soc.* 121:3004). Glycopolymers can induce affinity toward proteins such as lectins, and to viruses, due to multivalent recognition, known as the "cluster effect." (Lee, Y.C. and Lee, R.T., Eds. (1994) Neoglycoconjugates: Preparation and applications; Academic Press,
30 San Diego, CA; Roy, R. (1996) *Current Opinion in Structural Biology* 6:692-702.)

 The inability to generate GAGs through recombinant genetic engineering strategies, combined with the inherent complexity that has been associated with their direct chemical synthesis, has stimulated the development of a variety of biomimetic
35 synthetic approaches for the generation of carbohydrate-based macromolecules (Toshima, K. and Tatsuta, K. (1993) *Chem. Rev.* 93:1503; Roy, R. (1996) *Curr. Opin. Struct. Biol.* 6:692; and Roy, R. (1997) *Topics Curren. Chem.* 187:241).

Smaller oligosaccharide sequences may be responsible for the unique biological activities of the parent polysaccharides (Van Boeckel, C.A.A. et al. (1993) *Chem. Int. Ed. Engl.* 32:1671; Westerduin, P. et al. (1996) *Chem. Int. Ed. Engl.* 35:331; Petitou, M. et al. (1998), *Chem. Int. Ed.* 37:3009; Petitou, M. et al. (1999) *Nature* 398:417; Westman, J. et al. (1995) *Carbohydr. Chem.* 14:95; and Ornitz, D.M. et al. (1995) *Science* 268:432).

While advantages of such an approach exist, an inherent limitation is the loss of spatially controlled organization of multiple target saccharide sequences. Indeed the observation of enhanced protein binding affinity derived from multivalent oligosaccharide ligands has been termed the "cluster glycoside effect" (Lee, Y.C. In Synthetic Oligosaccharides: Indispensable Probes for the Life Sciences, ACS Symposium Series 560, Washington, D.C. 1994, Chapter 1, p. 6; Dimick, S.M. et al. (1999) *J. Am. Chem. Soc.* 121:10286; and Suda, Y. et al. (2000) *Polym. Prepr. (Am. Chem. Soc., Div. Polym. Chem.)* 41(2):1624).

An alternative glycomimetic strategy has consisted of the design of synthetic polymers that contain a hydrocarbon backbone with biologically active pendent saccharides. Fundamental studies on the synthesis and properties of model "glycopolymers" have proven to be useful in the characterization of specific biomolecular recognition processes (Miyata, T. and Nakamae, K. (1997) *Trends Polym. Sci.* 5:198).

Optimization of glycopolymer properties has required the utilization of biomolecular architectures that exhibit low fluctuations both in polymer size and in composition. A large variety of "living"/controlled polymerization techniques have recently emerged for this purpose, including ring-opening polymerization of sugar-substituted *N*-carboxyanhydrides (Aoi, K. et al. (1992) *Macromolecules* 25:7073; and Aoi, K. et al. (1994) *Macromolecules* 27:875); ring-opening metathesis polymerization (ROMP) of sugar-derivatized norbornenes (Fraser, C. and Grubbs, R.H. (1995) *Macromolecules* 28:7248; Nomura, K. and Schrock, R.R. (1996) *Macromolecules* 29:540); cationic polymerization of saccharide-carrying vinyl ethers (Minoda, M. et al. (1995) *Macromol. Symp.* 99:169; Yamada, K. et al. (1997) *J. Polym. Sci. Part A: Polym. Chem.* 35:751; and Yamada, K. et al. (1999) *Macromolecules* 32:3553); anionic polymerization of styrene derivatives containing monosaccharide residues (Loykulnant, S. et al. (1998) *Macromolecules* 31:9121; and Loykulnant, S. and Kirao, A. (2000), *Macromolecules* 33:4757); nitroxide-mediated free-radical polymerization of sugar-carrying styryl (Ohno, K. et al. (1998) *Macromolecules* 31:1064; and Ohno, K. et al. (1998) *Macromol. Chem. Phys.* 199:2193); and acryloyl (Ohno, K. et al. (1999) *Macromol. Chem. Phys.* 200:1619); monomers, as well as Atom Transfer Radical Polymerization (ATRP) of carbohydrate-based methacrylates (Ohno, K. et al. (1998) *J.*

Polym. Sci., Part A: Polym. Chem. 36:2473; Ejaz, M. et al. (2000) *Macromolecules* 33:2870; Bon, S.A.F. and Haddleton, D.M. (1999) *Polym. Prepr. (Am. Chem. Soc., Div. Polym. Chem.)* 40(2):248; and Marsh, A. et al. (1999) *J. Macromolecules* 32:8725).

5 Nevertheless, due to the incompatibility of hydroxyl groups from the saccharide moieties with either initiators or controlling agents, all of these approaches require the use of protected monomers and the subsequent deprotection of polymer chains to generate the desired glycopolymers.

10 All publications referred to herein are incorporated herein in their entirety to the extent not inconsistent herewith.

SUMMARY

This invention provides a glycopolymer composition comprising glycopolymer molecules having a polymer backbone; a first pendent unit comprising a linking group
15 connected to said polymer backbone and a saccharide moiety connected to said linking group; optionally a second pendent unit; a phenyl ring at a first end of the polymer backbone; and a cyanoxyl group at the second end of the polymer backbone.

20 These compounds are useful as, or as intermediates for making, bioactive glycopolymers for covalently or non-covalently binding to bioactive molecules. The molecules may be covalently bound to bioactive moieties through their end rings, or may bind to target bioactive molecules such as proteins, e.g. growth factors and cytokines, as well as viruses, cells and substrates, through their saccharide moieties, as is known to the art with respect to carbohydrate-mediated biomolecular recognition processes. The
25 molecules of this invention or end products lacking the cyanoxyl group synthesized using these products as intermediates, are useful in protein separation, cell culture, and drug-delivery systems, as well as in targeting for treatment of wound healing and other pathological conditions.

30 The polymer backbone is preferably a straight chain, but may also be branched.

The first pendent unit comprises a hydrocarbyl linking group connected to the backbone. Preferably the linking group contains about 3 to about 9 atoms, most preferably carbon atoms. It is preferably $(CH_2)_n$ where n is 3 to 9, and preferably is a straight chain,
35 but may also be an unsaturated and/or branched chain, may comprise additional moieties attached to the chain, and the chain may comprise heteroatoms, so long as formation of the polymer via free radical polymerization is not interfered with by such heteroatoms,

branching, and/or substituents. When the first pendent unit is made using an alkenyl-based glycomonomer, this helps lower the polydispersity index, thus it is preferred that an alkenyl-based glycomonomer be used.

5 The saccharide moieties are selected from the group consisting of monosaccharides, disaccharides, trisaccharides and oligosaccharides known to the art. Preferred saccharide moieties include *N*-acetyl-D-glucosamine, α - and β -*N*-acetyl-D-glucosamine-(1 \rightarrow 4)--D-glucuronic acid, pyranosides, lactose, and polylactose. The saccharides may be fully or partially sulfated, or may be unsulfated, i.e. may have SO₃ moieties replacing OH moieties.

 Preferably, the glycopolymer composition has a polydispersity index (molecular weight Mw/molecular number Mn) between about 1.1 and about 1.5.

15 The molecules of the glycopolymer composition preferably comprise between about 2 and about 1000 pendent saccharide moieties. When a second pendent is present, the sum of the number of saccharide moieties and second unit moieties is preferably no more than about 2000, more preferably no more than about 1000, and most preferably no more than about 100. The first and second units may be in *cis*- or *trans*- configuration with respect to each other. Consecutive first units may also be *cis*- or *trans*- to each other.

 The second unit preferably comprises about 3 to about 9 atoms, and may comprise substituents, branches, and hetero-atoms as does the linking group. It is preferably formed of an easily polymerizable hydrocarbyl monomer by copolymerization along with the first unit. Preferably the second unit is polymerized from acrylamide, acrylate, or alkenyl compounds, most preferably acrylamide. The molecules of this invention may comprise up to about 1000 of such second units. The second unit may function as a spacer, or may be derived from an easily-polymerizable monomer that promotes polymerization during the process of making the molecules of this invention.

30 The first and second units may be interspersed with each other in random order along the polymer backbone. As is known to the art, polymerization process parameters such as concentration of reactants, and temperature, will determine the statistical frequency and order of the first and second units.

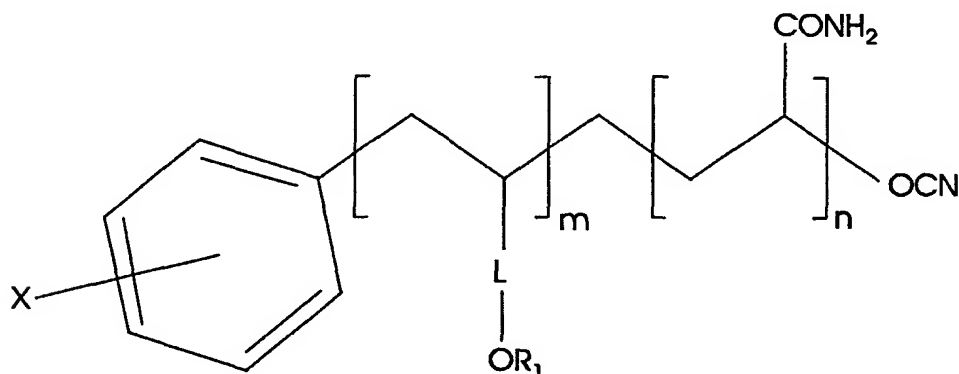
35 The phenyl ring may comprise at least one substituent which, depending on the electronic and steric effects of any substituent present, as is known to the art, may be

ortho, *meta* or *para*, to the position at which the ring attaches to the polymer backbone. Ring substituents can be hydrocarbyl, e.g. methoxy, alcohol, ether, amine, polyamine, sulfate, phosphate, nitrate, nitrite, halogen selected from the group consisting of chlorine, bromine and iodine, salts of the foregoing, and other substituents which do not interfere with formation of the polymer via free radical polymerization. Preferably the substituent is a *para* substituent, and preferably is chloro. In the process of making the compounds of this invention, the phenyl ring is derived from a corresponding diazonium salt.

The molecules of this invention may comprises hydrophobic and hydrophilic substituents as is known to the art. Preferably they are water soluble.

The molecules in solution are in equilibrium with free cyanoxyl radicals which may leave the terminal end of the polymer backbone, leaving it free to participate in further polymerization reactions.

In one embodiment, the molecules of this invention have the formula:



wherein X = H, Cl, NO₂ or OCH₃; L is a linker selected from the group of -(CH₂)_p- with p between 3 and about 9, or -COO(CH₂)₂-; OR₁ is selected from the group consisting of saccharide moieties; m is between 2 and about 1000; and n is between 0 and about 1000, wherein individual portions of the molecule designated by m and n may be in any order.

This invention also provides free radical polymerization methods for making the molecules of this invention. The method preferably comprises providing a cyanoxyl radical of an arenediazonium salt; providing glycomonomers comprising terminal vinyl

groups, and polymerizing the glycomonomers in the presence of said cyanoxyl radicals. The method may also include providing comonomers to form a second unit as described above, such as acrylamide molecules comprising vinyl groups. The method includes forming the glycomonomers comprising terminal vinyl groups by attaching them to alkenyl, acrylate or acylamide or other linking groups as described above. The cyanoxyl radicals may be made by contacting arenediazonium salts with cyanate anions. These reactions may be performed in aqueous or organic solution, and preferably are performed in aqueous solution, which has the advantage of not destroying functional groups as readily as organic solution.

The method is preferably performed at a temperature between about 50 and about 70 degrees C., and preferably, especially when acrylamide is used to provide the second unit, the glycomonomers used to form the first unit are provided at a ratio to the monomers used to form the second unit of about 1:4. These reaction conditions and ratio of monomers may vary, as will be appreciated by those skilled in the art, when it is desired to achieve a different density of saccharide moieties or other properties.

The term "hydrocarbyl" is used herein to refer generally to organic groups comprised of carbon chains to which hydrogen and optionally other elements are attached. CH₂ or CH groups and C atoms of the carbon chains of the hydrocarbyl may be replaced with one or more heteroatoms (i.e., non-carbon atoms). Suitable heteroatoms include but are not limited to O, S, P and N atoms. The term hydrocarbyl includes, but is not limited to alkyl, alkenyl, alkynyl, ether, polyether, thioether, ascorbate, aminoalkyl, hydroxylalkyl, thioalkyl, aryl and heterocyclic aryl groups, amino acid, polyalcohol, glycol, groups which have a mixture of saturated and unsaturated bonds, carbocyclic rings and combinations of such groups. The term also includes straight-chain, branched-chain and cyclic structures or combinations thereof. Hydrocarbyl groups are optionally substituted. Hydrocarbyl substitution includes substitution at one or more carbons in the group by moieties containing heteroatoms. Suitable substituents for hydrocarbyl groups include but are not limited to halogens, including chlorine, fluorine, bromine and iodine, OH, SH, NH, NH₂, COH, CO₂H, OR_a, SR_a, NR_aR_b, CONR_aR_b, where R_a and R_b independently are alkyl, unsaturated alkyl or aryl groups, sulfate, sulfite, phosphate, nitrate, carbonyl, and polyamine.

A "carbonyl compound" is any compound containing a carbonyl group (-C=O). The term "amine" refers to a primary, secondary, or tertiary amine group. A "polyamine" is a group that contains more than one amine group. A "sulfate" group is a salt of sulfuric

acid. Sulfate groups include the group $(\text{SO}_4)^{2-}$ and sulfate radicals. "Phosphates" contain the group PO_4^{3-} . "Glycols" are groups that have two alcohol groups per molecule of the compound.

5 The term "alkyl" takes its usual meaning in the art and is intended to include straight-chain, branched and cycloalkyl groups. The term includes, but is not limited to, methyl, ethyl, n-propyl, isopropyl, n-butyl, sec-butyl, isobutyl, tert-butyl, n-pentyl, neopentyl, 2-methylbutyl, 1-methylbutyl, 1-ethylpropyl, 1,1-dimethylpropyl, n-hexyl, 1-methylpentyl, 2-methylpentyl, 3-methylpentyl, 4-methylpentyl, 3,3-dimethylbutyl, 10 2,2-dimethylbutyl, 1,1-dimethylbutyl, 2-ethylbutyl, 1-ethylbutyl, 1,3-dimethylbutyl, n-heptyl, 5-methylhexyl, 4-methylhexyl, 3-methylhexyl, 2-methylhexyl, 1-methylhexyl, 3-ethylpentyl, 2-ethylpentyl, 1-ethylpentyl, 4,4-dimethylpentyl, 3,3-dimethylpentyl, 2,2-dimethylpentyl, 1,1-dimethylpentyl, n-octyl, 6-methylheptyl, 5-methylheptyl, 4-methylheptyl, 3-methylheptyl, 2-methylheptyl, 1-methylheptyl, 1-ethylhexyl, 15 1-propylpentyl, 3-ethylhexyl, 5,5-dimethylhexyl, 4,4-dimethylhexyl, 2,2-diethylbutyl, 3,3-diethylbutyl, and 1-methyl-1-propylbutyl. Alkyl groups are optionally substituted. Lower alkyl groups are C_1 - C_6 alkyl and include among others methyl, ethyl, n-propyl, and isopropyl groups.

20 The term "cycloalkyl" refers to alkyl groups having a hydrocarbon ring, preferably to those having rings of 3 to 7 carbon atoms. Cycloalkyl groups include those with alkyl group substitution on the ring. Cycloalkyl groups can include straight-chain and branched-chain portions. Cycloalkyl groups include but are not limited to cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, cyclooctyl, and cyclononyl. Cycloalkyl 25 groups can optionally be substituted.

 Aryl groups may be substituted with one, two or more simple substituents including, but not limited to, lower alkyl, e.g., methyl, ethyl, butyl; halo, e.g., chloro, bromo; nitro; sulfato; sulfonyloxy; carboxy; carbo-lower-alkoxy, e.g., carbomethoxy, carbethoxy; amino; mono- and di-lower-alkylamino, e.g., methylamino, ethylamino, 30 dimethylamino, methylethylamino; amido; hydroxy; lower-alkoxy, e.g., methoxy, ethoxy; and lower-alkanoyloxy, e.g., acetoxy.

 The term "unsaturated alkyl" group is used herein generally to include alkyl groups 35 in which one or more carbon-carbon single bonds have been converted to carbon-carbon double or triple bonds. The term includes alkenyl and alkynyl groups in their most general sense. The term is intended to include groups having more than one double or triple bond,

or combinations of double and triple bonds. Unsaturated alkyl groups include, without limitation, unsaturated straight-chain, branched or cycloalkyl groups. Unsaturated alkyl groups include without limitation: vinyl, allyl, propenyl, isopropenyl, butenyl, pentenyl, hexenyl, hexadienyl, heptenyl, cyclopropenyl, cyclobutenyl, cyclopentenyl, cyclopentadienyl, cyclohexenyl, cyclohexadienyl, 1-propenyl, 2-butenyl, 2-methyl-2-butenyl, ethynyl, propargyl, 3-methyl-1-pentynyl, and 2-heptynyl. Unsaturated alkyl groups can optionally be substituted.

Substitution of alkyl, cycloalkyl and unsaturated alkyl groups includes substitution at one or more carbons in the group by moieties containing heteroatoms. Suitable substituents for these groups include but are not limited to OH, SH, NH₂, COH, CO₂H, OR_c, SR_c, P, PO, NR_cR_d, CONR_cR_d, and halogens, particularly chlorines and bromines where R_c and R_d, independently, are alkyl, unsaturated alkyl or aryl groups. Preferred alkyl and unsaturated alkyl groups are the lower alkyl, alkenyl or alkynyl groups having from 1 to about 3 carbon atoms.

The term "aryl" is used herein generally to refer to aromatic groups which have at least one ring having a conjugated pi electron system and includes without limitation carbocyclic aryl, aralkyl, heterocyclic aryl, biaryl groups and heterocyclic biaryl, all of which can be optionally substituted. Preferred aryl groups have one or two aromatic rings.

"Carbocyclic aryl" refers to aryl groups in which the aromatic ring atoms are all carbons and includes without limitation phenyl, biphenyl and naphthalene groups.

"Aralkyl" refers to an alkyl group substituted with an aryl group. Suitable aralkyl groups include among others benzyl, phenethyl and picolyl, and may be optionally substituted. Aralkyl groups include those with heterocyclic and carbocyclic aromatic moieties.

"Heterocyclic aryl groups" refers to groups having at least one heterocyclic aromatic ring with from 1 to 3 heteroatoms in the ring, the remainder being carbon atoms. Suitable heteroatoms include without limitation oxygen, sulfur, and nitrogen. Heterocyclic aryl groups include among others furanyl, thienyl, pyridyl, pyrrolyl, N-alkyl pyrrolo, pyrimidyl, pyrazinyl, imidazolyl, benzofuranyl, quinolinyl, and indolyl, all optionally substituted.

"Heterocyclic biaryl" refers to heterocyclic aryls in which a phenyl group is substituted by a heterocyclic aryl group ortho, meta or para to the point of attachment of the phenyl ring to the decalin or cyclohexane. Heterocyclic biaryl includes among others groups which have a phenyl group substituted with a heterocyclic aromatic ring. The aromatic rings in the heterocyclic biaryl group can be optionally substituted.

"Biaryl" refers to carbocyclic aryl groups in which a phenyl group is substituted by a carbocyclic aryl group ortho, meta or para to the point of attachment of the phenyl ring to the decalin or cyclohexane. Biaryl groups include among others a first phenyl group substituted with a second phenyl ring ortho, meta or para to the point of attachment of the first phenyl ring to the decalin or cyclohexane structure. Para substitution is preferred. The aromatic rings in the biaryl group can be optionally substituted.

Aryl group substitution includes substitutions by non-aryl groups (excluding H) at one or more carbons or where possible at one or more heteroatoms in aromatic rings in the aryl group. Unsubstituted aryl, in contrast, refers to aryl groups in which the aromatic ring carbons are all substituted with H, e.g. unsubstituted phenyl ($-C_6H_5$), or naphthyl ($-C_{10}H_7$). Suitable substituents for aryl groups include among others, alkyl groups, unsaturated alkyl groups, halogens, OH, SH, NH_2 , COH, CO_2H , OR_e , SR_e , NR_eR_f , $CONR_eR_f$, where R_e and R_f independently are alkyl, unsaturated alkyl or aryl groups. Preferred substituents are OH, SH, OR_e , and SR_e where R_e is a lower alkyl, i.e., an alkyl group having from 1 to about 3 carbon atoms. Other preferred substituents are halogens, more preferably chlorine or bromine, and lower alkyl and unsaturated lower alkyl groups having from 1 to about 3 carbon atoms. Substituents include bridging groups between aromatic rings in the aryl group, such as $-CO_2-$, $-CO-$, $-O-$, $-S-$, $-P-$, $-NH-$, $-CH=CH-$ and $-(CH_2)_\rho-$ where ρ is an integer from 1 to about 5, and preferably $-CH_2-$. Examples of aryl groups having bridging substituents include phenylbenzoate. Substituents also include moieties, such as $-(CH_2)_\rho-$, $-O-(CH_2)_\rho-$ or $-OCO-(CH_2)_\rho-$, where ρ is an integer from about 2 to 7, as appropriate for the moiety, which bridge two ring atoms in a single aromatic ring as, for example, in a 1, 2, 3, 4-tetrahydronaphthalene group. Alkyl and unsaturated alkyl substituents of aryl groups can in turn optionally be substituted as described *supra* for substituted alkyl and unsaturated alkyl groups.

The terms "alkoxy group" and "thioalkoxy group" (also known as mercaptide groups, the sulfur analog of alkoxy groups) take their generally accepted meaning. Alkoxy groups include but are not limited to methoxy, ethoxy, n-propoxy, isopropoxy, n-butoxy, sec-butoxy, isobutoxy, tert-butoxy, n-pentyloxy, neopentyloxy, 2-methylbutoxy,

1-methylbutoxy, 1-ethyl propoxy, 1,1 -dimethylpropoxy, n-hexyloxy, 1-methylpentyloxy, 2-methylpentyloxy, 3-methylpentyloxy, 4-methylpentyloxy, 3,3-dimethylbutoxy, 2,2-dimethoxybutoxy, 1,1-dimethylbutoxy, 2-ethylbutoxy, 1-ethylbutoxy, 1,3-dimethylbutoxy, n-pentyloxy, 5-methylhexyloxy, 4-methylhexyloxy, 3-methylhexyloxy, 2-methylhexyloxy, 1-methylhexyloxy, 3-ethylpentyloxy, 2-ethylpentyloxy, 1-ethylpentyloxy, 4,4-dimethylpentyloxy, 3,3-dimethylpentyloxy, 2,2-dimethylpentyloxy, 1,1-dimethylpentyloxy, n-octyloxy, 6-methylheptyloxy, 5-methylheptyloxy, 4-methylheptyloxy, 3-methylheptyloxy, 2-methylheptyloxy, 1-methylheptyloxy, 1-ethylhexyloxy, 1-propylpentyloxy, 3-ethylhexyloxy, 5,5-dimethylhexyloxy, 4,4-dimethylhexyloxy, 2,2-diethylbutoxy, 3,3-diethylbutoxy, 1-methyl-1-propylbutoxy, ethoxymethyl, n-propoxymethyl, isopropoxymethyl, sec-butoxymethyl, isobutoxymethyl, (1-ethyl propoxy)methyl, (2-ethylbutoxy)methyl, (1-ethylbutoxy)methyl, (2-ethylpentyloxy)methyl, (3-ethylpentyloxy)methyl, 2-methoxyethyl, 1-methoxyethyl, 2-ethoxyethyl, 3-methoxypropyl, 2-methoxypropyl, 1-methoxypropyl, 2-ethoxypropyl, 3-(n-propoxy)propyl, 4-methoxybutyl, 2-methoxybutyl, 4-ethoxybutyl, 2-ethoxybutyl, 5-ethoxypentyl, and 6-ethoxyhexyl. Thioalkoxy groups include but are not limited to the sulfur analogs of the alkoxy groups specifically listed *supra*.

"Optional" or "optionally" means that the subsequently described event or circumstance may or may not occur, and that the description includes instances where said event or circumstance occurs and instances in which it does not. For example, "optionally substituted phenyl" means that the phenyl radical may or may not be substituted and that the description includes both unsubstituted phenyl radicals and phenyl radicals wherein there is substitution.

"Contacting" reaction components with each other refers to providing a medium and/or reaction chamber in which the reaction components are placed together so that they can react with each other. Preferably, the reaction components are suspended or dissolved in a carrier fluid which is a liquid medium. "Maintaining reaction components in contact" means keeping the components together in such a way that they can react with each other.

Saccharides include straight chain or cyclic saccharides, i.e., mono-, di- and poly-, straight chain and cyclic saccharides. They may have optional substituents that do not interfere with polymerization or binding to the target protein or other biologically active component, such as the substituents set forth above for hydrocarbyls. Straight chain saccharides that are useful in this invention include but are not limited to those molecules

with a chain of 5 or 6 carbon atoms with one or more -OH groups attached, and either an aldehyde or ketone group. Cyclic saccharides are saccharides that are in a ring form.

Disaccharides are compounds wherein two monosaccharide groups are linked.

Polysaccharides (also referred to as oligosaccharides) are compounds wherein more than
5 two monosaccharide groups are linked.

Water soluble groups or hydrophilic groups includes groups that, when included as a substituent, imparts substantial solubility in water to the compound. Water soluble groups include, but are not limited to alcohols; polyalcohols; straight chain or cyclic
10 saccharides; amines and polyamines; sulfate groups; phosphate groups; ascorbate groups; alkyl chains optionally substituted with -OH at any position; glycols, including polyethylene glycols, and polyethers.

The term "biologically active" means capable of effecting a change in a living
15 organism or component thereof.

The cyanoxyl glycopolymers of this invention may be further modified to remove the cyanoxyl moiety for use in medical applications. Compositions for use in medical applications are preferably hydrophilic and are highly pure or are purified to a highly
20 pure state such that they may be injected into a human patient. They may comprise or be modified to comprise water soluble groups or hydrophilic groups which when included as substituents, impart substantial solubility in water to the compound. Water soluble groups include, but are not limited to alcohols; polyalcohols; straight chain or cyclic saccharides; amines and polyamines; sulfate groups; phosphate groups; ascorbate groups;
25 alkyl chains optionally substituted with -OH at any position; glycols, including polyethylene glycols, and polyethers comprising atoms available for forming hydrogen bonds in aqueous solutions.

The polymerizable linkers used to facilitate polymerization of the molecules of this
30 invention may be ethylene, ethylene glycol, oxyethylene, methylene glycol, trimethylene glycol, vinylpyrrolidones, and derivatives thereof, all as is known to the art. Polyethylene glycol can be rendered monofunctionally activated by forming an alkylene ether group at one end. The alkylene ether group may be any suitable alkoxy radical having 1-6 carbon atoms, for example, methoxy, ethoxy, propoxy, 2-propoxy, butoxy, hexyloxy, and the like.

35

Preferably, the compositions of this invention are "nonimmunogenic," i.e. produce no appreciable immunogenic or allergic reaction when injected or otherwise implanted into the body of a human subject.

5 The terms "cytokine" and "growth factor" are used to describe biologically active molecules and active peptides (which may be either naturally occurring or synthetic) which aid in healing or regrowth of normal tissue including growth factors and active peptides. Cytokines can incite local cells to produce new collagen or tissue, or they can attract cells to a site in need of correction. Cytokines include interferons (IFN), tumor
10 necrosis factors (TNF), interleukins, and colony stimulating factors (CSFs). Growth factors include osteogenic factor extract (OFE), epidermal growth factor (EGF), transforming growth factor (TGF) alpha, TGF-beta. (including any combination of TGF-beta.s), TGF-B 1, TGF-B2, platelet derived growth factor (PDGF-AA, PDGF-AB, PDGF-BB), acidic fibroblast growth factor (FGF), basic FGF, connective tissue activating
15 peptides (CTAP), beta.-thromboglobulin, insulin-like growth factors, erythropoietin (EPO), nerve growth factor (NGF), bone morphogenic protein (BMP), osteogenic factors, and the like. Glycopolymers made using the compositions of this invention bound to cytokines or growth factors may serve as effective controlled release drug delivery means. By varying the chemical linkage between the glycosaminoglycan and the synthetic
20 polymer, it is possible to vary the effect with respect to the release of the cytokine or growth factor. For example, when an "ester" linkage is used, the linkage is more easily broken under physiological conditions, allowing for sustained release of the growth factor or cytokine from the matrix. However, when an "ether" linkage is used, the bonds are not easily broken and the cytokine or growth factor will remain in place for longer periods of
25 time with its active sites exposed, providing a biological effect on the natural substrate for the active site of the protein. It is possible to include a mixture of conjugates with different linkages so as to obtain variations in the effect with respect to the release of the cytokine or growth factor, i.e., the sustained release effect can be modified to obtain the desired rate of release.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 shows an ^1H NMR spectrum of a 5(C-9)/AM glycopolymer sample of this invention. The spectrum was recorded at room temperature with a Varian INOVA 400 spectrometer with a magnetic field strength of 400 MHz. The sample concentration was
35 10 mg/mL and D_2O was used as the solvent and internal standard, 4.8 ppm.

Figure 2 shows an ^1H NMR (D_2O) spectrum of a 17(C-3)/AM glycopolymer sample of this invention (17(C-3)/AM = 1/16 (mol) 17(C-3): 20 wt%, $M_{n,\text{NMR}} = 3700$ g/mol, $M_{n,\text{SEC}} = 4800$ g/mol).

5 Figure 3 shows a SEC/RJ/LLS chromatogram of a 17(C-3)/AM glycopolymer sample ($M_n = 112,100$ g/mol, $M_w/M_n = 1.20$).

DETAILED DESCRIPTION

This invention discloses synthesis of a series of model *N*-acetyl-D-glucosamine-
10 carrying unprotected glycomonomers and their use in the synthesis of glycompolymers which serve as glycosaminoglycan-mimetic architectures. Some glycosaminoglycan-mimetic architectures are related to heparan sulfate. Well-characterized glycopolymers serve as useful model systems for investigating protein-carbohydrate interactions relevant to endothelial regeneration and angiogenesis. The design of biomaterials
15 capable of promoting these physiological processes may have significant impact in the areas of wound repair and tissue regeneration, as well as other phenomena influenced by glycosaminoglycans. Tailored glycopolymers are contributing to the progress of glycotecology, including the design of non-thrombogenic biomaterials that enhance tissue regeneration and wound healing responses. Tailored glycopolymers also provide
20 model systems for investigating protein-carbohydrate interactions relevant to endothelial regeneration.

This invention broadens the family of vinyl monomers that are controllably polymerizable by using cyanoxyl-mediated free-radical polymerization. Both nonsulfated
25 and sulfated alkene- as well as acrylate-derivatized monosaccharides are polymerizable using this methodology.

Cyanoxyl-mediated free-radical polymerization utilizes cyanoxyl persistent radicals as chain-growth moderators of the statistical copolymerization of acrylamide
30 with either alkene- or acrylate-based glycomonomers. In association with a cyanoxyl-mediated polymerization strategy, acrylic monosaccharides are utilized to generate homo-glycopolymers with some degree of control. Cyanoxyl ($\bullet\text{OC}\equiv\text{N}$)-mediated free-radical polymerization provides an effective and versatile method for engineering a

diverse array of water-soluble glycopolymers with high saccharide contents and low polydispersity indexes preferably $1.1 < M_w/M_n < 1.6$.

Cyanoxyl-mediated free-radical polymerization can be conducted in aqueous solution, is tolerant of a broad range of functional groups (such as -OH, NH₂, -COOH, and others known to the art), yields low-polydispersity polymers, and is applicable to the synthesis of block and graft copolymers. Cyanoxyl-mediated copolymerization is an efficient approach for preparing glycopolymers with high monosaccharide contents and polymer compositions in close agreement with expected values.

The examples hereof demonstrate the synthesis of a series of mono- and disaccharide-containing glycopolymers by two different free radical processes. In the first methodology, cyanoxyl persistent radicals ($\bullet\text{OC}\equiv\text{N}$) are effectively employed as moderators of the statistical copolymerization of acrylamide (AM) with either mono- or disaccharide-based ω -alkenyl glycomonomers. The statistical cyanoxyl-mediated copolymerization of acrylamide with either nonsulfated or sulfated alkene-derivatized unprotected glycomonomers is a practical and effective method for engineering a diverse array of glycopolymers with high saccharide contents and low polydispersity indexes. The results of this approach are compared to those obtained via the classical free-radical ammonium peroxodisulfate (APS)/N,N,N',N'-tetmethylethylenediamine (TMEDA) initiating system. The examples hereof illustrate the anticipated absence of control over copolymerization using the classical APS/TMEDA initiating system. It is not possible to synthesize a large variety of water-soluble glycopolymers with high carbohydrate contents and low polydispersity indexes ($1.1 < M_w/M_n < 1.5$) with classical free-radical polymerization such as using APS/TMEDA initiation.

Cyanoxyl persistent radicals impart control to the polymerization by scavenging growing radicals and forming dormant species that can reversibly undergo homolytic bond cleavage, in a manner similar to that reported for nitroxyl-mediated processes. In the presence of cyanoxyl radicals that are unable to initiate polymerization, a low stationary concentration of macroradicals is maintained which prevents bimolecular irreversible termination from occurring to the extent observed in classical free-radical mechanisms.

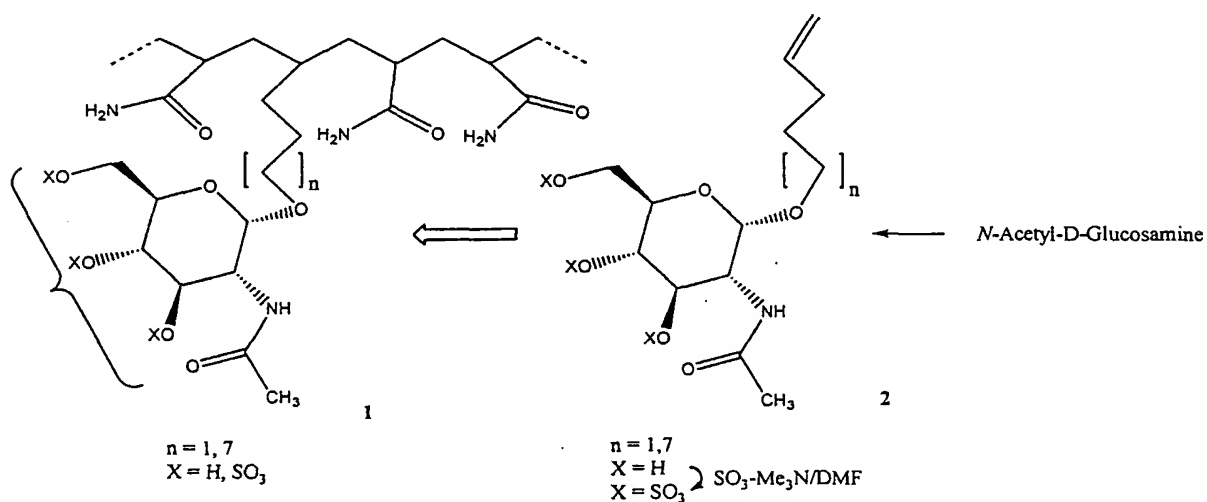
Further, the use of $\bullet\text{OC}\equiv\text{N}$ radicals generated at moderate temperatures (25-70°C) avoids unintended thermal polymerization of monomers.

As a starting point for our synthetic studies, model vinyl-derivatized glycomonomers were synthesized from *N*-acetyl-D-glucosamine. However, a more efficient strategy was developed that yielded glycomonomers in a single step.

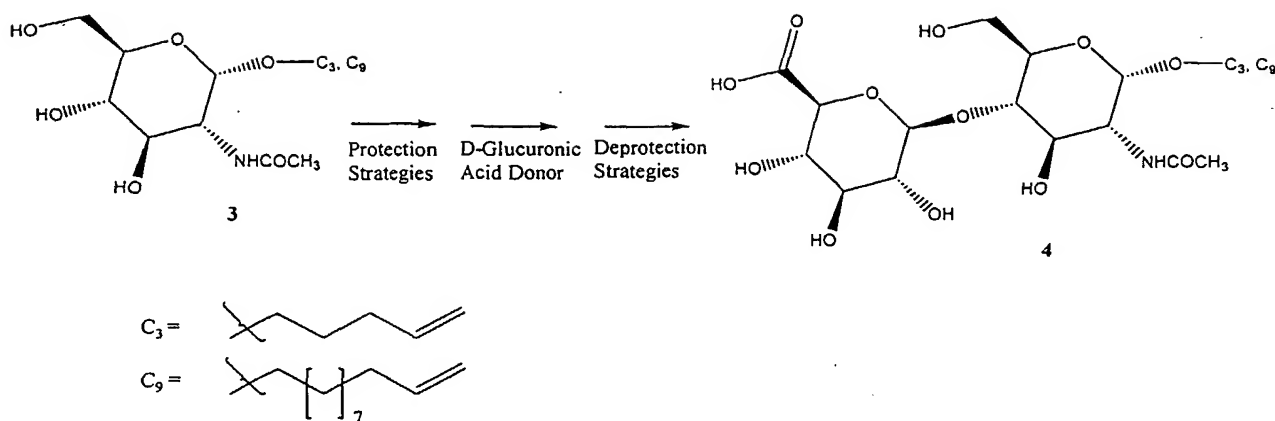
Schemes 1 and 2 demonstrate the synthesis of heparan sulfate-related glycomonomers viz. monosaccharides (compound 2) and disaccharides (compound 4) which bear a glycosamine moiety at the reducing end containing a vinyl polymerizable double bond.

The synthetic strategies are based on preparing the glucuronic acid-glucosamine (GluA-GluNAc) sequence utilizing different protecting and deprotecting strategies.

Nonsulfated alkene-derivatized unprotected glycomonomers were directly synthesized from *N*-acetyl-D-glucosamine.



Scheme 1.



Scheme 2.

Example 1 and Scheme 3 demonstrate the synthesis of model glycomonomers from *N*-acetyl-D-glucosamine. **Example 2** and Scheme 4 demonstrate the chemoselective sulfation of glycomonomer hydroxy groups. **Example 3**, Scheme 5, and Table 1 demonstrate cyanoxyl radical-mediated glycomonomer copolymerization. **Example 4** and Table 2 demonstrate the preparation of acrylamide-based glycopolymers using the classical APS/TMEDA initiating system. **Example 5** and Scheme 6 demonstrate the use of cyanoxyl radicals as moderators of the statistical copolymerization of acrylamide with the synthesized glycomonomers which provides a mechanism for controlling both polymer molar mass and architectural features based upon block structures. **Example 6** and Scheme 7 demonstrate the synthesis of nonsulfated and sulfated glycopolymers. **Example 7**, Scheme 8, and Table 3 demonstrate cyanoxyl ($\bullet\text{OC}\equiv\text{N}$) persistent radicals used as moderators of the statistical copolymerization of AM with the synthesized glycomonomers. **Example 8** demonstrates classical free-radical copolymerizations of glycomonomers and AM using APS/TMEDA. **Example 9** demonstrates the synthesis of nonsulfated *N*-acetyl-D-glucosamine-carrying glycomonomer. **Example 10** demonstrates the synthesis of nonsulfated lactose-based glycomonomer. **Example 11** demonstrates the preparation of sulfated glycomonomers. **Example 12** demonstrates the synthesis of nonsulfated GlcUA/GlcNAc bases glycomonomers. **Example 13**, Scheme 9, and Table 4 demonstrate cyanoxyl-mediated free-radical copolymerization of AM with alkene-derivatized unprotected glycomonomers. **Example 14** and Table 5 demonstrate classical free-radical copolymerizations of glycomonomers and AM using APS/TMEDA. **Example 15** and Scheme 10 demonstrate the synthesis of nonsulfated alkene-derivatized

glycomonomers. **Example 16** and Scheme 11 demonstrate the synthesis of nonsulfated acrylic monomers. **Example 17** and Scheme 12 demonstrate the preparation of sulfated glycomonomers. **Example 18** and Scheme 13 demonstrate the statistical polymerization of alkene-derivatized glycomonomers and acrylamide initiated by $\text{ClC}_6\text{H}_4\text{N}\equiv\text{N}^+\text{BF}_4^-$ /NaOCN. **Example 19** and Table 7 demonstrate homopolymerization of acrylic glycomonomers initiated by $\text{ClC}_6\text{H}_4\text{N}\equiv\text{N}^+\text{BF}_4^-$ /NaOCN.

Regardless of the glycomonomer used (nonsulfated/sulfated, short/long spacer arm), it is remarkable that the carbohydrate contents as well as the molar masses increased with monomer conversion while the polydispersity indexes remained below 1.5. When an initial ratio of glycomonomer to AM of 1/4 was employed, a copolymer that displayed monosaccharide content in close agreement with that expected was obtained after 16 h of reaction. Weight proportions of sugar residues as high as 50% were thus reached. Nevertheless, a higher carbohydrate content in the resulting copolymer was associated with an increase in the polydispersity index. Thus, some loss of control over the copolymerization process sometimes occurs in the presence of increasing amounts of glycomonomer. This is probably due to the innate low chemical reactivity of the unactivated vinyl group in the saccharide monomer. It is also noteworthy that spacer-arm length of the glycomonomer influenced the polymerization behavior. Indeed, the amount of incorporated carbohydrate was increased with decreasing spacer-arm length.

Notably, saccharide contents as well as molar masses increased with monomer conversion while polydispersity indexes remained below 1.5. Regardless of the glycomonomer used (mono/disaccharide-based, nonsulfated/sulfated), an initial ratio GM/AM of 1/4, associated with a copolymerization time of 16 h, permitted access to a copolymer that displayed a saccharide content in close agreement with that expected. Samples exhibiting sugar compositions as high as 69 wt % were thus obtained. Nonetheless, copolymers with higher carbohydrate contents were associated with an increase in polydispersity indexes. This may be attributable to some loss of control over the copolymerization process in the presence of increasing amounts of ω -alkenyl glycomonomers, considering the innate low chemical reactivity of the unactivated vinyl group in these saccharide monomers.

Cyanoxyl radicals were generated by an electron-transfer reaction between cyanate anions ($\bullet\text{OC}\equiv\text{N}$), from a NaOCN aqueous solution, and *p*-chlorobenzene-diazonium salts ($\text{ClC}_6\text{H}_4\text{N}\equiv\text{NBF}_4^-$), that were previously prepared *in situ* through a diazotization reaction of *p*-chloroaniline in water (**Example 18**, Scheme 13). In addition to cyanoxyl persistent radicals, aryl-type active radicals were simultaneously produced, and only the latter species is capable of initiating chain growth. A large variety of water-soluble glycopolymers were generated by varying the nature of the ω -alkenyl glycomonomer (nonsulfated/sulfated, C-3/C-9 spacer arm), as well as the initial molar ratio of glycomonomer (GM) to acrylamide (AM) in the statistical cyanoxyl-mediated copolymerization of both comonomers (Table 6). Statistical copolymers were characterized by ^1H NMR spectroscopy (see Figure 2, as an example), as well as by size-exclusion chromatography (SEC) coupled with both refractive index (RI) and LLS detection systems (see Figure 3, as an example). ^1H NMR made it possible to verify the absence of residual comonomers, particularly glycomonomer, in the purified glycopolymers. The ratio of resonance signal intensities due to methyl protons from *N*-acetyl groups (2.0 ppm) and methine protons (2.1-2.4 ppm) from the hydrocarbon skeleton allowed determination of monosaccharide content. It is noteworthy that the spacer-arm length of ω -alkenyl glycomonomer influenced the copolymerization behavior. Indeed, the proportion of incorporated carbohydrate in the final copolymer increased with decreasing spacer-arm length from *n*-nonyl (C-9) to *n*-propyl (C-3). This suggests that C-3 spacer-armed glycomonomers have a higher reactivity than their C-9 homologues.

The degree of control over the macromolecular structure is significantly better with cyanoxyl-mediated free-radical polymerization than that observed by resorting to classical free-radical processes. Polydispersity indexes for all copolymers remained below 1.5, which defines a theoretical lower limit for a conventional free-radical mechanism. Moreover, regardless of the glycomonomer used and the initial GM/AM molar ratio, monosaccharide contents in the resulting glycopolymers as well as their molar masses increased with comonomer conversion. Yet, it should be stressed that contrary to a truly “living”/controlled radical polymerization, cyanoxyl-terminated samples of predetermined molar masses cannot be designed by ending the polymerization reaction at a certain conversion. Indeed, actual molar masses ($M_{n\text{SEC}}$ obtained from SEC/RI/LLS) were systematically much higher than theoretical values ($M_{n\text{th}} = M_0 \times ([M]_0/[I]_0) \times \pi$, where M_0

stands for the molar mass of a monomeric unit and π for monomer conversion, assuming a complete initiator efficiency of $f = 1$). We presume that a large proportion of the moderately reactive phenyl-type initiating radicals are lost in irreversible primary termination reactions at the initial stages of the polymerization process, thus decreasing initiator efficiency (f). Values as low as 0.1 were indeed estimated for this parameter by taking the ratio of theoretical to actual molar masses ($f = M_{n,th}/M_{n,SEC}$). On the other hand, it is also interesting to point out that the use of an initial GM/AM molar ratio of 1/4 enabled the design of a copolymer that exhibited a monosaccharide content in close agreement with that expected, after 16 h of reaction. Mass compositions of sugar residues as high as 50 wt % were thus reached. Nonetheless, a higher carbohydrate content in the resulting copolymers was associated with a broadening of the molar mass distributions (M_w/M_n). This is probably attributable to some loss of control over the copolymerization process in the presence of increasing amounts of ω -alkenyl glycomonomers, considering the innate low chemical reactivity of the unactivated vinyl group in these saccharide monomers.

The low level of polymerizability associated with alkene-derivatized monosaccharides in free-radical processes, and particularly in cyanoxyl-mediated polymerization, implies two major limitations. First, that the yield of copolymers with AM is low even after a reaction time of 16 h and second, that homopolymers cannot be derived from the polymerization of these glycomonomers, as confirmed by further investigation. Consequently, nonsulfated and sulfated acrylate-based glycomonomers were synthesized. As summarized in Table 7, cyanoxyl-mediated homopolymerization of these monosaccharides could be achieved with some degree of control. This is illustrated by the low polydispersity indexes ($1.13 < M_w/M_n < 1.56$) observed for nonsulfated and sulfated homo-glycopolymers. Samples of different molar masses were also prepared by varying either monomer conversion or the initial ratio of monomer to initiator concentrations ($[M]_0/[I]_0$). Acrylic glycomonomers were also copolymerized with AM to yield low-polydispersity (~ 1.5) statistical copolymers with saccharide contents as high as 75 wt %. Due to the much higher reactivity of acrylic monomers compared with that of their ω -alkenyl counterparts, higher conversions were reached within 4 h, or even 1.5 h in some instances (Table 2). However, polydispersity indexes of both nonsulfated and sulfated acrylic glycopolymers were generally higher than those obtained for alkene-

derivatized homologues. Presumably, this arises from the non-negligible contribution of irreversible bimolecular termination reactions that characterizes free-radical polymerization of acrylates. The presence of cyanoxyl radicals as chain-growth moderators, nonetheless, makes it possible to minimize the extent of these side reactions.

5

All solvents and reagents were purchased from commercial sources and were used as received, unless otherwise noted. Deionized water with a resistivity of 18 MΩcm was used as solvent in all polymerization reactions.

10

All reactions were performed in flame-dried glassware under an atmosphere of dry argon. The reaction medium solutions were evaporated under reduced pressure with a rotary evaporator, and the residue was chromatographed on a silica gel (230-400 mesh) column. Analytical thin-layer chromatography (TLC) was performed on Whatman silica gel aluminum backed plates of 250 μm thickness on which spots were visualized with

15

UV light or charring the plate before and/or after dipping in a H₂SO₄-EtOH mixture.

Melting point (mp) measurements were performed with a Thomas Hoover melting point apparatus in open capillary tubes and were uncorrected. Mass spectra (MS/FAB) were obtained at an ionizing voltage of 70 eV. Optical rotations were determined with a Perkin Elmer-2 GIMC polarimeter. ¹H and ¹³C NMR spectra were recorded at room

20

temperature with a Varian INOVA 400 spectrometer (magnetic field strengths of 400 MHz and 100 MHz for ¹H and ¹³C NMR analyses respectively). In all cases, the sample concentration was 10 mg/mL, and the appropriate deuterated solvent was used as

internal standard. The size-exclusion chromatography (SEC) equipment comprised a Waters model 510 HPLC pump, a Waters Ultrahydrogel 250 column, and a Wyatt

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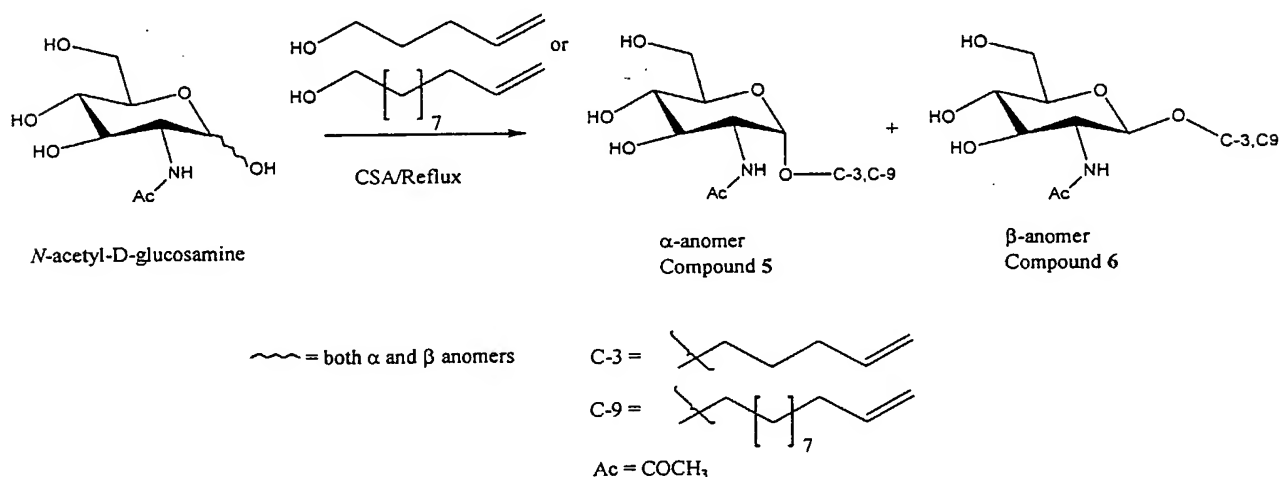
Technology Optilab 903 refractometer. The eluent consisted of a 0.1M NaNO₃ deionized water solution containing 0.05 wt % sodium azide at a flow rate of 0.7 mL/min. The actual molar masses of the glycopolymer samples were determined from the response of the DAWN F (Wyatt Technology) multiangle laser light-scattering (LLS) detector that was connected to the outlet of the SEC apparatus.

30

EXAMPLES

Example 1

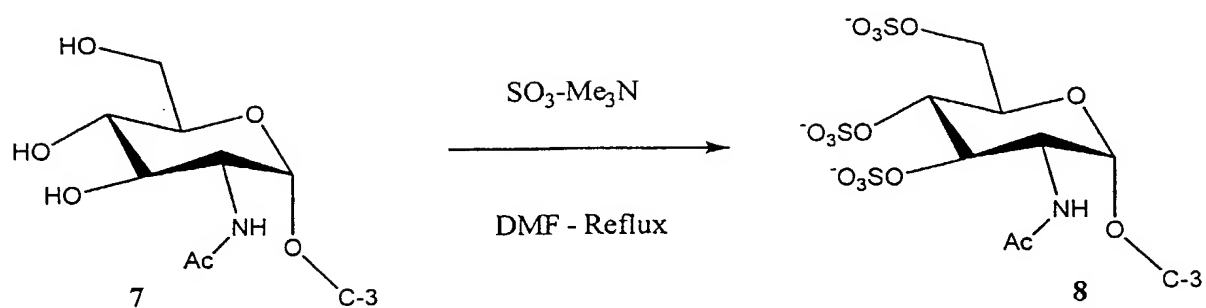
Model glycomonomers were synthesized from *N*-acetyl-D-glucosamine. *N*-acetyl-D-glucosamine was treated with 4-penten-1-ol and ω -undecenol in the presence of 10-camphor sulphonic acid as catalyst. This reaction produced the α,β -anomeric mixture of the corresponding spacer-arm glycomonomer (Scheme 3). Anomers were successfully separated by column chromatography (SiO₂, CHCl₃/MeOH (9/1)) and were characterized by ¹H and ¹³C NMR spectroscopy. Yields of the α - and β -anomers were 31 % and 11%, respectively.



Scheme 3. Synthesis of Glycomonomers, Compounds 5 and 6

Example 2

Chemoselective sulfation of hydroxy groups with α -anomer was effected using $\text{SO}_3 \text{NMe}_3$ complex (scheme 4). The product was purified by anion-exchange and size exclusion chromatography and characterized by ^1H and ^{13}C NMR, as well as by mass spectral analysis.



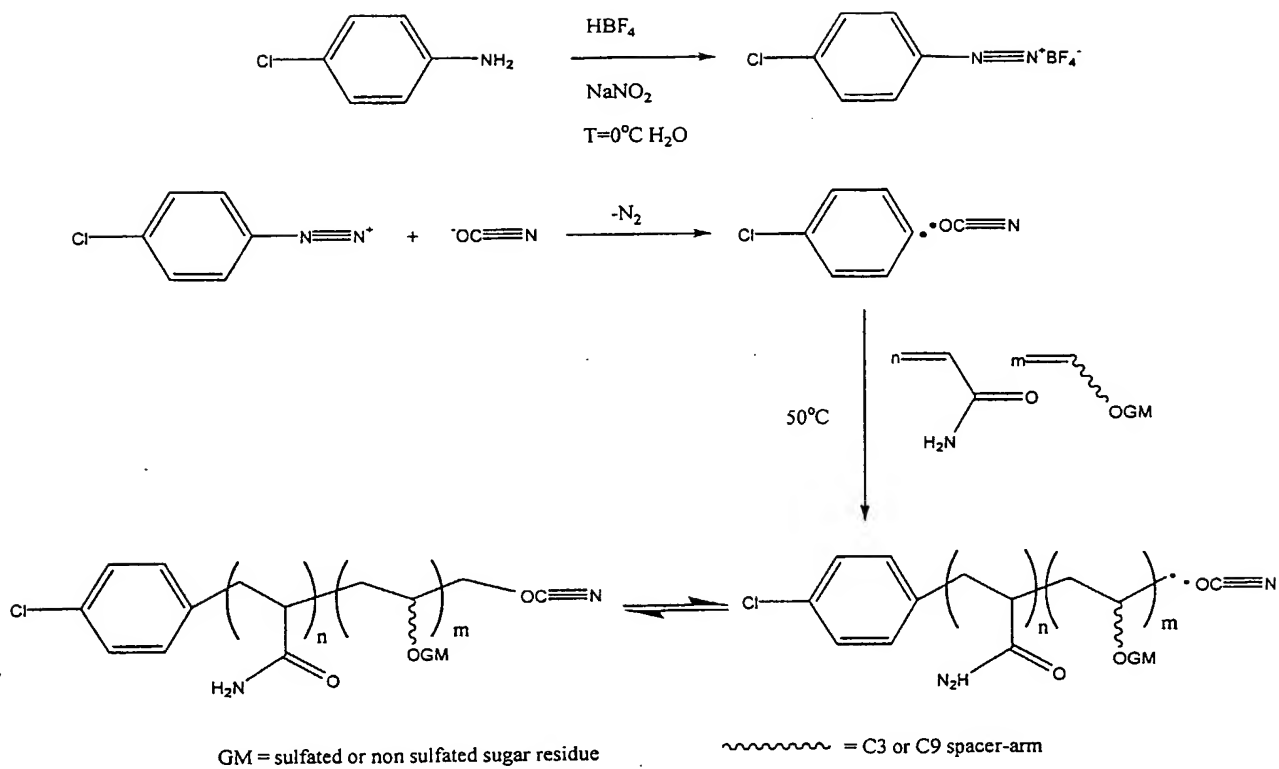
Scheme 4. Synthesis of Sulfated Glycomonomer, Compound 8

Example 3.

Cyanoxyl radicals were generated *in situ* by an electron-transfer reaction between cyanate anions ($\text{OC}\equiv\text{N}$) and *para*-chloro-benzenediazonium cations ($\text{ClC}_6\text{H}_4\text{N}\equiv\text{N}^+$).

Arenediazonium salts were prepared in water through a diazotization reaction of *para*-chloro-aniline (Scheme 5). The copolymerizations were performed at 50°C or 70°C using $\text{ClC}_6\text{H}_4\text{N}\equiv\text{N}^+\text{BF}_4^-/\text{NaOCN}$ as the initiating system. Results are shown in Table 1. The statistical copolymers obtained were isolated by precipitation in a tenfold excess of methanol and characterized by ^1H NMR spectroscopy (Figure 1). The carbohydrate content of the copolymers was determined by using the ratio of the intensities of the resonance signals due to the methyl protons of *N*-acetyl groups from monosaccharide residues (2.0 ppm) and to the methine protons (between 2.1 and 2.4 ppm) of the main chain.

Regardless of the glycomonomer used (non sulfated/sulfated, short spacer-arm/long spacer-arm), when an initial ratio of glycomonomer to AM of 1/4 was employed, a copolymer that displayed a monosaccharide content in close agreement with that expected was obtained after 16 hours of reaction. Weight proportion of sugar residues as high as 50% were thus reached. Notably, an increase in the polymerization time (40 hours) did not significantly improve either the incorporation rate of the glycomonomer or the yield. However, an increase of the polymerization temperature from 50°C to 70°C did enhance the reactivity of the glycomonomer such that its incorporation rate was improved. It is of interest that spacer-arm length of the glycomonomer did influence polymerization behavior. Indeed, the amount of incorporated carbohydrate was increased with decreasing spacer-arm length. It is also noteworthy that the incorporation of the glycomonomer was improved at higher GM/AM ratios.



5 Scheme 5. Copolymerization of AM with glycomonomers in the presence of cyanoxyl persistent radicals.

Table 1. Experimental Conditions and Results of the Free-Radical Copolymerizations of AM with Miscellaneous Glycomonomers using $\text{ClC}_6\text{H}_4\text{N}\equiv\text{N}^+\text{BF}_4^-/\text{NaOCN}$ as initiating system

Glycomonomer (GM)	Monomer Ratio GM/AM mol.	Time (h)	Yield (%)	Polymer Composition ^1H NMR (mol.)	Saccharide Content (wt%)
5(C-3) ^{a)}	1/4	1.5	10	1/7 117	37.7
		16	30	1/5	49.9
	1/20	1.5	23	1/90	4.3
		16	30	1/70	5.3
		40	35	1/65	5.9
5(C-3) ^{b)}	1/20	1.5	20	1/70	5.4
		16	26	1/50	6.9
5(C-9) ^{a)}	1/4	1.5	15	1/16	25.2
		16	20	1/6	45.6
	1/20	1.5	21	1/200	2.5
		16	29	1/100	5.0
7 ^{a)}	1/4	1.5	21	1/10	43.6
		16	35	1/6	54.5
	1/20	1.5	35	1/93	7.4
		16	51	1/58	11.3

a) T=50°C ;

b) T=70°C ;

$[\text{M}]_0 = [\text{GM}]_0 + [\text{AM}]_0 = 1\text{M}$;

$[\text{I}]_0 = [\text{ClC}_6\text{H}_4\text{N}\equiv\text{N}^+\text{BF}_4^-]_0 = [\text{NaOCN}]_0 = 2 \times 10^{-2}\text{M}$

Example 4

Classical free-radical copolymerizations of glycomonomers and AM were carried out using APS and TMEDA as the initiating system. TMEDA accelerates the homolytic scission of APS yielding sulfate (SO_4^{2-}), hemiTMEDA ($((\text{CH}_3)_2\text{NCH}_2\text{CH}_2(\text{CH}_3)\text{NCH}_2\bullet)$), and hydroxyl ($\bullet\text{OH}$) radical species. Copolymerization was performed in a dilute solution of water/methanol (1/1) [despite reports by Nishimura, S.-L; Matsuoka, K.; Kurita, K. (1990) *Macromolecules* 23:4182 and Nishimura, S.-L; Matsuoka, K.; Furuike, T.; Ishii, S.; Kurita, K. (1991) *Macromolecules* 24:4236 that 5(C-9) glycomonomer cannot undergo a copolymerization reaction in water due to its poor solubility]. The reaction medium was homogeneous and the polymerization proceeded efficiently at both room temperature and 50°C. Results of copolymerizations attempted with 5(C-3) or 5(C-9) glycomonomers are summarized in Table 2. After a defined reaction time, the medium was dialyzed against deionized water and freeze-dried to afford water-soluble statistical copolymers with yields up to 70%. Varying the initial GM/AM monomer ratio modified the sugar content in the macromolecules. Regardless of the conditions used in this series of experimental reactions, the glycomonomer incorporation rate was significantly lower than that obtained using cyanoxyl-mediated polymerization. Additionally, by increasing the temperature of the copolymerization from 25°C to 50°C, the monosaccharide content was increased, though yield was decreased. This might be attributable to a higher loss of active centers at 50°C. However, this reduction in yield could be offset by increasing the initial monomer concentration.

Table 2. Experimental Conditions and Results of the Free-Radical Copolymerization of Compound 5(C-3) or Compound 5(C-9) Glycomonomer and AM in the presence of APS and TMEDA

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[M] ₀ mol/L)	[I] ₀ (mol/L)	Time (h)	GM/AM (mol.)	Yield (%)	Polymer Composition ¹ H NMR mol.	Saccharide Content (wt%)
0.2 ^{a)}	0.002	16	1/4	37	1/75	6.6
0.2 ^{a)}	0.002	16	1/10	24	1/150	3.6
0.2 ^{a)}	0.002	16	1/20	42	1/350	1.6
0.2 ^{b)}	0.002	40	1/4	17	1/16	24.1
0.2 ^{b)}	0.002	40	1/10	08	1/16	24.2
0.2 ^{b)}	0.002	40	1/20	15	1/44	10.7
0.4 ^{a)}	0.004	16	1/4	50	1/36	12.8
0.4 ^{a)}	0.004	40	1/4	35	1/40	11.6
0.4 ^{b)}	0.004	16	1/4	27	1/32	14.2
0.2 ^{c)}	0.002	16	1/4	14	1/26	13.3
1 ^{c)}	0.02	1.5	1/4	67	1/14	26.7
		16		73	1/11	34.3
1 ^{d)}	0.02	1.5	1/4	08	1/11	22.0
		16		10	1/8	28.0

[M]₀=[GM]₀+ [AM]₀; [I]₀ = [APS]₀ = [TMEDA]₀

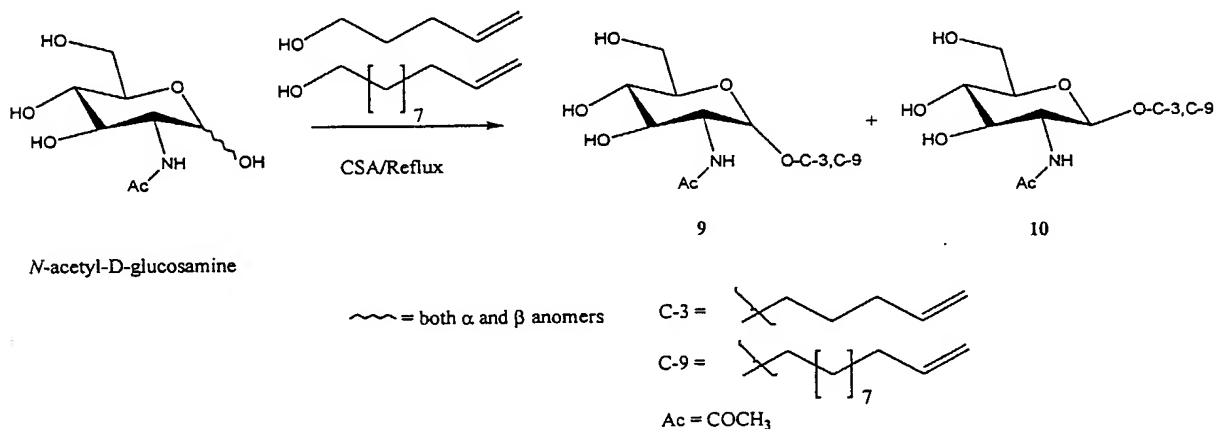
a) GM=5(C-9), T=25°C ; b) GM=5(C-9), T=50°C; c) GM=5(C-3), T=25°C

d) GM=5(C-3), T=50°C

Example 5

Synthesis of Nonsulfated and Sulfated Glycomonomers

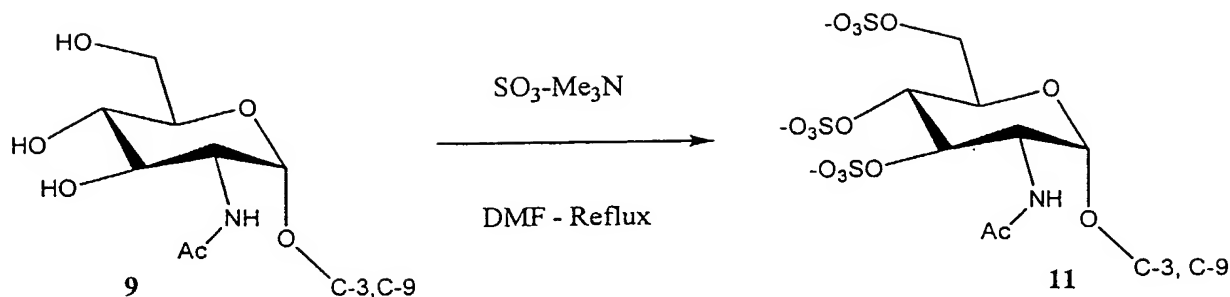
N-acetyl-D-glucosamine, on treatment with 4-penten-1-ol or 10-undecen-1-ol in the presence of 10-camphorsulfonic acid (CSA) as catalyst, provided the α,β -anomeric mixture of the corresponding spacer arm containing glycomonomer (Scheme 6). Anomers 9 and 10 were successfully separated by column chromatography (5102, CHC131MeOH (911)) and were characterized by ^1H and ^{13}C NMR spectroscopy. Yields of α - and β -anomers were 31% and 11%, respectively.



Scheme 6. Synthesis of Nonsulfated Glycomonomers, 9 and 10

Example 6

Chemoselective sulfation of hydroxy groups on the α -anomer (9) was effected using the $\text{SO}_3\text{-NMe}_3$ complex (Scheme 7). The product (11) was purified by anion-exchange and size-exclusion chromatography and characterized by ^1H and ^{13}C NMR, as well as by mass spectral analysis.



Scheme 7. Synthesis of Sulfated Glycomonomers, 11

5

Example 7

Synthesis of Nonsulfated and Sulfated Glycopolymers

- 10 Cyanoxyl radicals were readily generated *in situ* by an electron-transfer reaction between cyanate anions ($\text{OC}\equiv\text{N}^-$) and *p*-chlorobenzenediazonium cations ($\text{ClC}_6\text{H}_4\text{N}\equiv\text{N}^+$). The arenediazonium salts were previously prepared in water through diazotization reaction of *p*-chloroaniline (Scheme 8). The results of copolymerizations performed at 50 °C using $\text{ClC}_6\text{H}_4\text{N}\equiv\text{N}^+\text{BF}_4^-/\text{NaOCN}$ as the initiating system are shown in Table 3. The statistical
- 15 copolymers obtained were isolated by precipitation in a 10-fold excess of methanol and characterized by ^1H NMR spectroscopy (see Figure 1, as an example), as well as by size-exclusion chromatography (SEC) coupled with a refractive index (RI) detector and a multiangle laser light-scattering (LLS) detector. The monosaccharide content of the copolymers was determined by taking the ratio of the intensities of the resonance signals due
- 20 to the methyl protons of *N*-acetyl groups from carbohydrate residues (2.0 ppm) and to the methine protons (between 2.1 and 2.4 ppm) of the main chain.

Table 3. Experimental Conditions and Results of Free-Radical Copolymerization of AM with Miscellaneous Glycomonomers Using $\text{ClC}_6\text{H}_4\text{N}\equiv\text{N}+\text{BF}_4/\text{NaOCN}$ as Initiating System^a

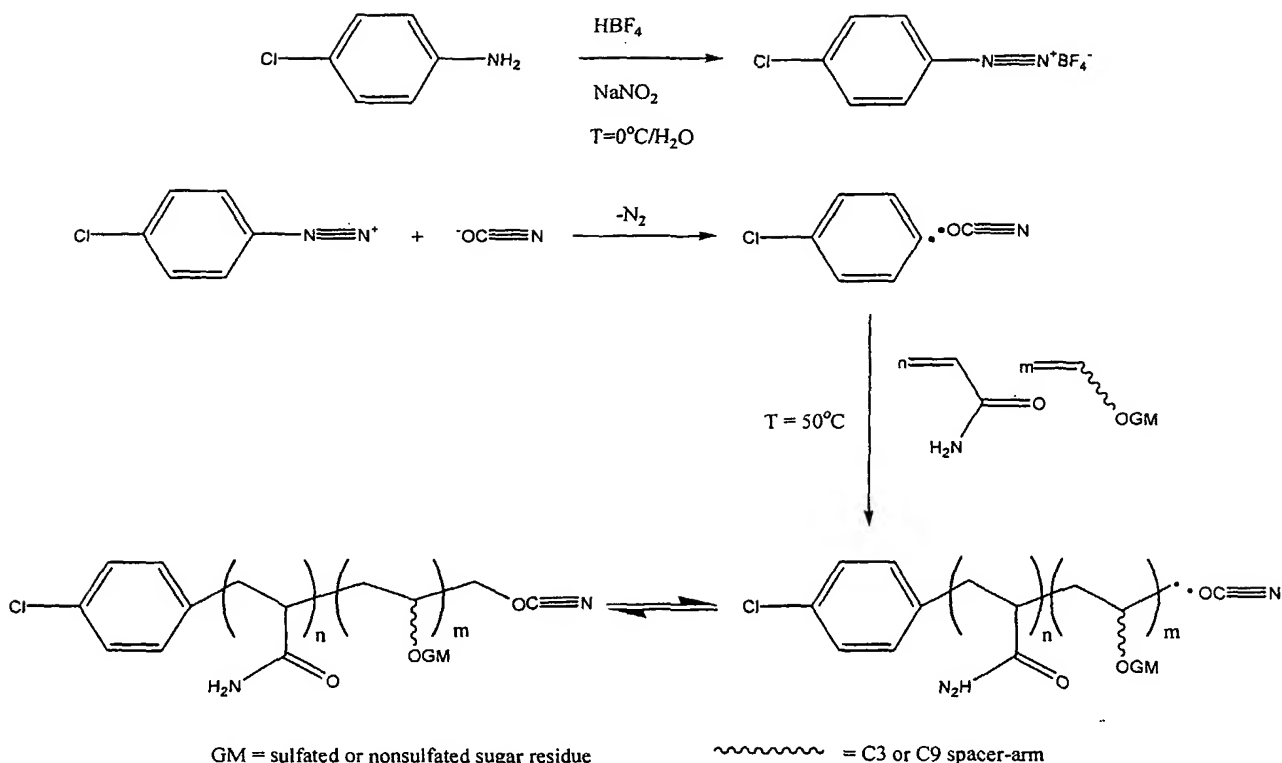
glycomonomer (GM)	monomer ratio GM/AM (mol)	Time (h)	yield ^b (%)	polymer composition ^c (mol)	monosaccharide content (wt %)	M_n^d (g/mol)	M_w/M_n SEC
9(C-3)	1/4	1.5	10	1/7	37.7	24 100	1.46
		16	30	1/5	49.9	43 000	1.47
	1/20	1.5	23	1/90	4.3	94 000	1.17
9(C-9)		16	30	1/70	5.3	112 100	1.20
	1/4	1.5	15	1/16	25.2	43 400	1.25
		16	20	1/6	45.6	99 300	1.45
11(C-3)		1.5	21	1/166	3.1	25 800	1.14
	1/20	16	29	1/100	5.0	28 200	1.24
	1/4	1.5	21	1/10	43.6	16 100	1.13
11(C-9)		16	35	1/6	54.5	57 300	1.37
	1/20	1.5	35	1/93	7.4	25 400	1.10
		16	51	1/58	11.3	47 200	1.29
11(C-9)	1/4	16	26	1/10	45.4	57 200	1.20
	1/20	16	11	1/42	17.0	16 300	1.17

^a T = 50 °C, $[\text{M}]_0 = [\text{GM}]_0 + [\text{AM}]_0 = 1\text{M}$, $[\text{I}]_0 = [\text{ClC}_6\text{H}_4\text{N}\equiv\text{N}^+\text{BF}_4]_0 = [\text{NaOCN}]_0 = 2 \times 10^{-2} \text{ mol/L}$.

^b Total conversion of both comonomers as determined by gravimetry.

^c Molar ratio of monosaccharide to acrylamide monomeric units in the resulting copolymer as determined by ^1H NMR.

^d M_n obtained from SEC (eluent, water, column, Waters Ultrahydrogel 250) equipped with a LLS detector (Wyatt Technology).



5

Scheme 8. Copolymerization of AM with glycomonomers in the presence of cyanoxyl persistent radicals

10 Example 8

In a comparative analysis, classical free-radical copolymerizations of glycomonomers and AM were carried out using ammonium peroxodisulfate (APS) and *N,N,N',N'*-tetramethylethylenediamine (TMEDA) as the initiating system. TMEDA accelerates the homolytic scission of APS yielding sulfate ($\text{SO}_4^{\bullet-}$), hemiTMEDA ($(\text{CH}_3)_2\text{NCH}_2\text{CH}_2(\text{CH}_3)\text{NCH}_2\bullet$), and hydroxyl ($\bullet\text{OH}$) radical species. However, we were able to obtain a copolymer by performing the copolymerization in a dilute solution of water/THF (1/1). The reaction medium was homogeneous, and the polymerization proceeded efficiently at room temperature. Utilizing identical experimental conditions as those used for cyanoxyl-mediated processes ($[\text{M}]_0 = 1$ mol/L, $[\text{I}]_0 = 2 \times 10^{-2}$ mol/L, GM/AM $\approx 1/4$), the resulting glycopolymers exhibited lower monosaccharide contents (up to 30 wt %) and especially higher molar masses and polydispersity indexes (1.7-2.0). Moreover, increasing the polymerization time from 1.5 to 16

h increased the polydispersity index but otherwise had little effect on either monosaccharide content or molar mass.

Example 9

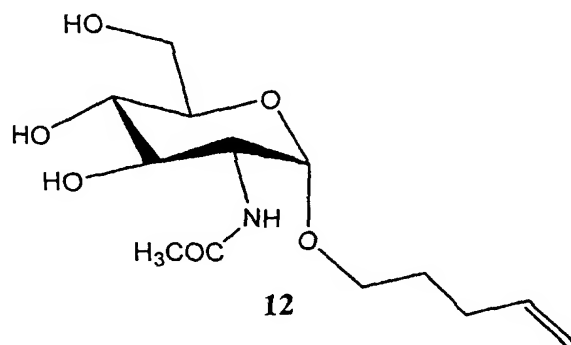
5

Synthesis of Nonsulfated N-acetyl-D-glucosamine-Carrying Glycomonomer

As a starting point for our synthetic studies, a nonsulfated alkene-derivatized unprotected monosaccharide (compound 12) was directly synthesized from N-acetyl-D-glucosamine. We resorted to the 10-camphorsulfonic acid (CSA)/reflux method as a facile means of yielding the desired C-3 spacer arm-containing glycomonomer. Thus, N-acetyl-D-glucosamine was refluxed at 110 °C for 9 h with a catalytic amount of CSA and a large excess of ω -pentenyl alcohol to provide a mixture of α - and β -anomers in an average 60-70% crude yield. The latter compounds were separated by silica gel column chromatography (eluent: CHCl₃/MeOH (9/1)) with a ratio (α / β) of 3/1, and were characterized by ¹H and ¹³C NMR, as well as by mass spectrometry and polarimetry.

10

15



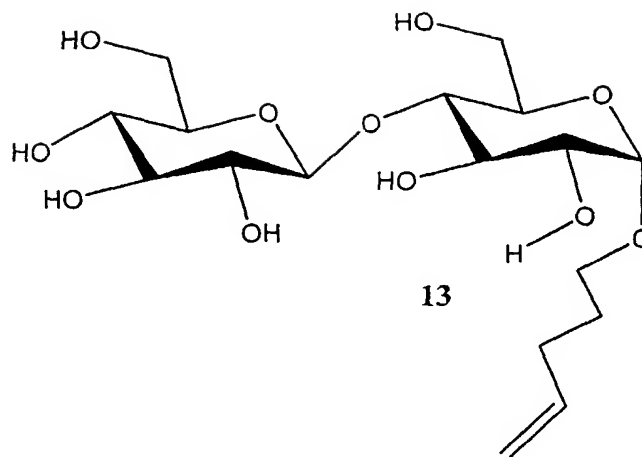
20

Example 10

Synthesis of Nonsulfated Lactose Based Glycomonomer

Lactose and ω -pentenyl alcohol were subjected to the aforementioned CSA/reflux methodology to yield a nonsulfated alkene-derivatized unprotected lactose (compound 13).

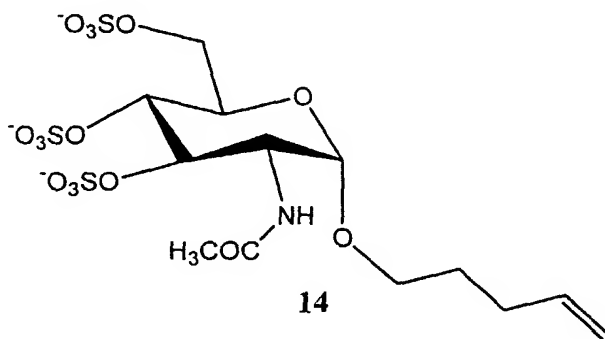
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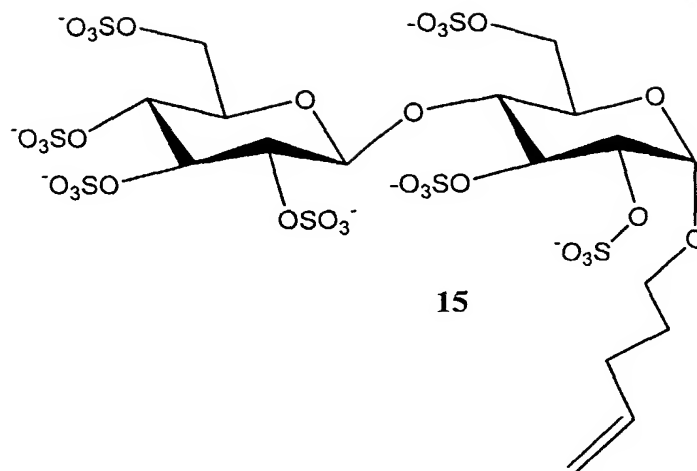


Example 11

5 Preparation of Sulfated Glycomonomers

Chemoselective sulfation of all hydroxyl groups on α -anomers, namely compounds **12** and **13**, was achieved by treating them with $\text{SO}_3\text{-NMe}_3$ complex at 60°C in DMF. This treatment actually resulted in crude mixtures of unreacted and sulfated derivatives. Hence, these mixtures were passed through a diethylaminoethyl (DEAE)-sephacel anion-exchange resin column, eluting first with a 10mol/L sodium phosphate buffer (pH ~ 7.0), whereby the unreacted nonsulfated compounds were removed. The sulfated homologues were then eluted with a 1 mol/L NaCl buffer (pH - 7.0), and recovered as mixtures of their trisodium salts with an excess of NaCl. These eluates were finally passed through a Trisacryl size-exclusion resin column for isolation of the pure sulfated products (compounds **14** and **15**) in 30-35 % yield.

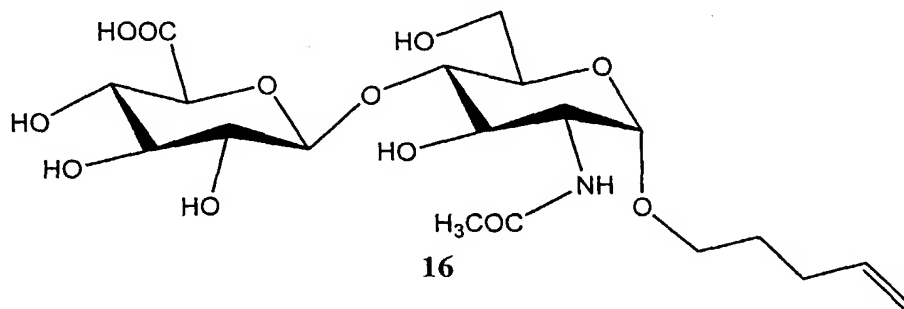




Example 12

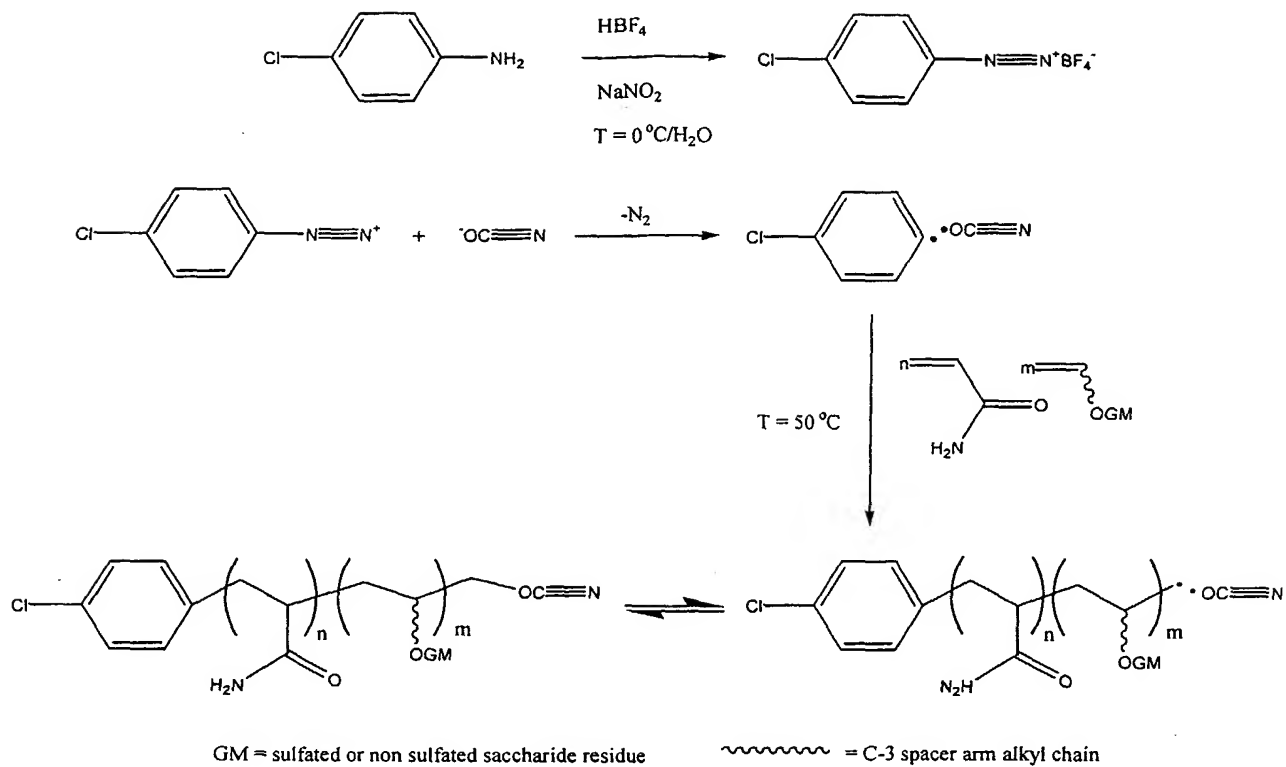
5 *Synthesis of Nonsulfated GlcUA/GlcNAc Based Glycomonomer*

The target disaccharide sequence β -GlcUA-(1 \rightarrow 4)- α -GlcNAc was derived from the glycosidation of the fully protected glucuronic acid (GlcUA) donor having an anomeric leaving group with the α -alkenyl *N*-acetyl-D glucosamine-containing (GlcNAc) acceptor which possesses an unprotected hydroxyl group at the 4-position. Successive protection/deprotection strategies were utilized to synthesize donor and acceptor molecules. All hydroxyl functionalities were deprotected in the resulting protected disaccharide and the carboxylic ester from the donor moiety was converted to the corresponding acid to afford the expected alkene-derivatized disaccharide (compound 16). This glycomonomer was thoroughly characterized by ^1H NMR, ^{13}C NMR and mass spectrometry.



Example 13

Cyanoxyl radicals were readily generated by an electron-transfer reaction between cyanate anions ($\bullet\text{OC}\equiv\text{N}$) from a NaOCN aqueous solution, and *p*-chlorobenzenediazonium salts ($\text{ClC}_6\text{H}_4\text{N}\equiv\text{N}^+\text{BF}_4^-$) that were previously prepared *in situ* through a diazotization reaction of *p*-chloroaniline in water. In addition to cyanoxyl persistent radicals, aryl-type active radicals were simultaneously produced, and only the latter species were able to initiate chain growth (Scheme 9). A variety of water-soluble glycopolymers were prepared by varying the nature of the α -alkenyl glycomonomer (nonsulfated/sulfated, mono-/disaccharide-based), as well as the initial molar ratio of glycomonomer (GM) to AM in the statistical copolymerization of both comonomers performed at 50°C (Table 4). These statistical copolymers were isolated by precipitation in a 10-fold excess of methanol and characterized by ^1H NMR spectroscopy, as well as by size-exclusion chromatography (SEC) coupled with both refractive index and laser light-scattering detectors. The absence of residual comonomers, and especially of glycomonomer in the purified glycopolymers, was checked by ^1H NMR. The ratio of resonance signal intensity of methyl protons of *N*-acetyl groups from sugar moieties (2.0 ppm) to that of methine protons from the hydrocarbon skeleton (2.1-2.4 ppm) enabled the determination of saccharide content for glycopolymers containing *N*-acetyl-D-glucosamine residues. As to lactose-based samples, elemental analysis was used to assess their carbohydrate composition.



Scheme 9. Cyanoxyl-Mediated Free-Radical Copolymerization of AM with Alkene-Derivatized Unprotected Glycomonomers

5

Table 4. Statistical Free-Radical Copolymerization of AM with ω -Alkenyl Mono- or Disaccharide-Based Unprotected Glycomonomers Using $\text{ClC}_6\text{H}_4\text{N}\equiv\text{N}^+\text{BF}_4^-/\text{NaOCN}$ as Initiating System^a

GM	monomer ratio GM/AM (mol)	time (h)	yield ^b (%)	polymer composition GM/AM (mol)	saccharide content (wt%)	M_n (g/mol)	M_w/M_n SEC
1	1/4	1.5	10	1/7	38	24,100	1.46
1	1/4	16	30	1/5	50	43,000	1.47
1	1/20	1.5	23	1/90	04	94,000	1.17
1	1/20	16	30	1/70	05	112,100	1.2
2	1/4	1.5	21	1/10	44	16,100	1.13
2	1/4	16	35	1/6	55	57,300	1.37
2	1/20	1.5	35	1/93	07	25,400	1.10
2	1/20	16	51	1/58	11	47,200	1.29
3	1/20	16	15	1/30	16	28,800	1.31
4	1/4	16	30	1/6	69	38,000	1.50
5	1/20	16	15	1/27	20	9,200	1.21

^a $T=50^\circ\text{C}$, $[\text{GM}]_0 + [\text{AM}]_0 = 1 \text{ mol/L}$, $[\text{I}]_0 = [\text{ClC}_6\text{H}_4\text{N}\equiv\text{N}^+\text{BF}_4^-]_0 = [\text{NaOCN}]_0 = 0.02 \text{ mol/L}$. ^b Total conversion of comonomers as determined by gravimetry.

Example 14

In a comparative study, classical free-radical copolymerizations of glycomonomers and AM were performed by using APS/TMEDA as the initiating system. TMEDA actually accelerates the homolytic scission of APS yielding sulfate (SO_4^{2-}), hemiTMEDA ($((\text{CH}_3)_2\text{NCH}_2\text{CH}_2(\text{CH}_3)\text{NCH}_2\bullet)$), and hydroxyl ($\bullet\text{OH}$) radical species. The reaction medium was homogeneous and the polymerization proceeded smoothly at room temperature (Table 5). Utilizing otherwise identical experimental conditions as those used for cyanoxyl-mediated processes ($[\text{M}]_0 = 1 \text{ mol/L}$, $[\text{I}]_0 = 2 \times 10^{-2} \text{ mmol/L}$), the resulting glycopolymers exhibited lower saccharide contents (maximum: 43 wt %) and especially higher molar masses and polydispersity indexes (1.6-2.0). Moreover, increasing the polymerization time from 1.5 to 16 h increased the polydispersity index, but otherwise had little influence on either saccharide content or molar mass.

Table 5. Statistical Free-Radical Copolymerization of AM with ω -Alkenyl Mono- or Disaccharide-Based Unprotected Glycomonomers Using APS/TMEDA as Initiating System^a

GM	monomer ratio GM/AM (mol)	time (h)	yield ^b (%)	polymer composition GM/AM (mol)	saccharide content (wt%)	M _n (g/mol)	M _w /M _n SEC
1	1/4	1.5	67	1/14	22	133,000	1.63
1	1/4	16	73	1/11	28	146,000	1.73
3	1/4	16	83	1/10	38	140,000	1.78
3	1/20	16	80	1/48	11	156,000	2.08
4	1/4	16	50	1/18	43	70,500	1.59
4	1/20	16	70	1/60	18	80,000	1.89
5	1/20	16	69	1/20	25	54,400	1.62
5	1/20	16	66	1/75	08	44,500	1.60

^a T=25 °C, [M]₀ = [GM]₀ + [AM]₀ = 1 mol/L, [I]₀ = [APS]₀ = [TMEDA]₀ = 0.02 mol/L.

^b Total conversion of comonomers as determined by gravimetry.

Example 15

Synthesis of nonsulfated alkene-derivatized glycomonomers

Hence, *N*-acetyl-D-glucosamine was refluxed with a catalytic amount of CSA and a large excess of either α -pentenyl or ω -undecenyl alcohol to provide a mixture of α - and β -anomers (Scheme 10, 17(C-3) or 17(C-9), and 18(C-3) or 18(C-9), respectively) in an average 60-70 % crude yield. The latter compounds were separated by column chromatography with a ratio (α/β) of 3/1 for both cases. The yield was improved by varying the temperature and reaction time. The α - and β -anomeric configurations of the separated products were determined from ¹H NMR data, namely J_{1,2} coupling constants of 3.6 Hz and 8.0 Hz, respectively. To a mixture of *N*-acetyl-D-glucosamine (10 g) and either 4-penten-1-ol or 10-undecen-1-ol (large excess, ~50-75 mL) was added a catalytic amount of 10-camphorsulphonic acid (CSA) (~400 mg) and the mixture was refluxed at 110°C for 9h. The reaction mixture was then cooled, neutralized with triethylamine, and the excess of alcohol was removed under vacuum. Moreover, the rest of the solid mass was rinsed with hot petroleum ether to remove the rest of ω -alkenyl alcohol. The residue was purified by column chromatography using a chloroform/methanol (97/3) mixture to afford the expected α - and β -anomers. (Scheme 10, 17(C-3) or 17(C-9), and 18(C-3) or 18(C-9), respectively).

n-Pentenyl 2-Acetamido-2-deoxy- α -D-glucopyranoside, 17(C-3)

Yield: 46 %. mp: 146-148°C. $[\alpha]_D^{20} = +142.2^\circ$ (c 1.3, MeOH). ^1H NMR ($\text{CDCl}_3 + 5\%$ CD_3OD), δ_{ppm} : 1.68 (m, 2H), 2.02 (s, 3H, NHCOCH_3), 2.12 (m, 2H), 3.41 (dt, 1H, $J = 6.4$ and 10 Hz), 3.68 (m, 4H), 3.78 (d, 1H, $J = 3.2$ and 12.4 Hz), 4.00 (dd, 1H, $J = 3.2$ and 12.4 Hz), 4.02 (m, 1H), 4.77 (d, 1H, $J_{1,2} = 3.6$ Hz, *H-1*), 5.01 (m, 2H, $\text{CH}_2=$), 5.80 (m, 1H, CH=), 6.61 (d, 1H, $J = 8.8$ Hz, NHCOCH_3). ^{13}C NMR ($\text{CDCl}_3 + 5\%$ CD_3OD), δ_{ppm} : 22.9, 28.4, 30.2, 53.6, 61.2, 67.2, 70.1, 71.6, 72.4, 97.3, 114.9, 137.9, 171.7. MS/FAB, m/z : 289 (M^+).

10 *n*-Undecenyl 2-Acetamido-2-deoxy- α -D-glucopyranoside, 17(C-9)

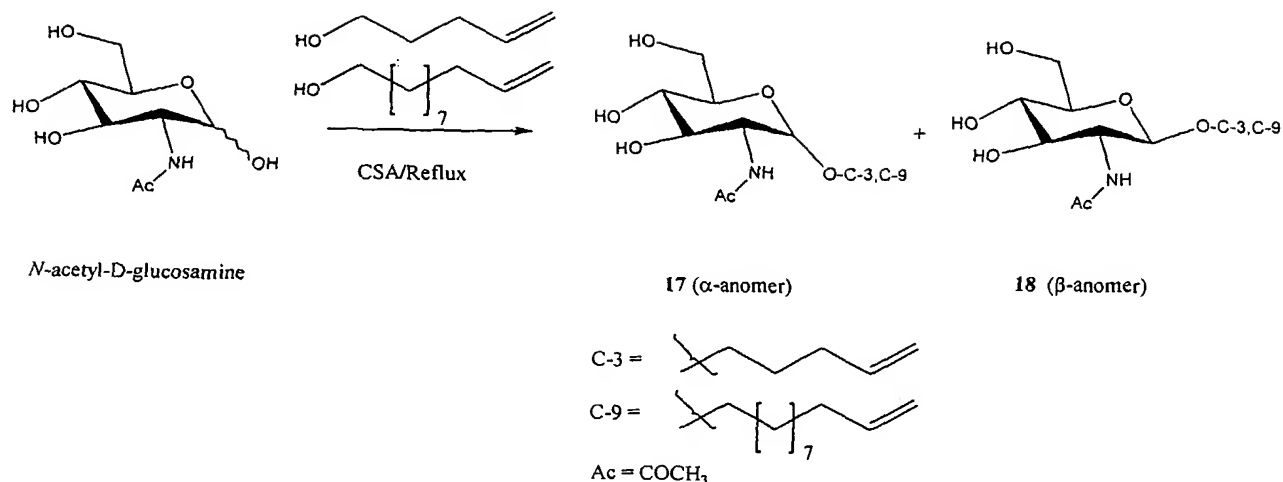
Yield: 50 %. mp: 152-154°C. $[\alpha]_D^{20} = -9.0^\circ$ (c 0.2; MeOH). ^1H NMR ($\text{CDCl}_3 + 5\%$ CD_3OD), δ_{ppm} : 1.34 (m, 9H), 1.54 (m, 2H), 2.02 (m and s, 5H), 3.33 (m, 1H), 3.68 (m, 4H), 3.77 (br d, 1H, $J = 9.6$ Hz), 3.92 (br d, 1H, $J = 9.6$ Hz), 4.06 (m, 1H), 4.78 (d, 1H, $J_{1,2} = 3.6$ Hz, *H-1*); 4.95 (m, 2H, $\text{CH}_2=$), 5.80 (m, 1H, CH=), 6.43 (d, 1H, $J = 8.8$ Hz, NHCOCH_3). ^{13}C NMR ($\text{CDCl}_3 + 5\%$ CD_3OD), δ_{ppm} : 23.3, 26.1, 28.9, 29.1, 29.3, 29.4, 29.5, 29.6, 33.8, 53.7, 61.2, 68.0, 69.9, 71.6, 72.8, 97.4, 114.1, 139.1, 171.7. MS/FAB, m/z : 373 (M^+).

n-Pentenyl 2-Acetamido-2-deoxy β -D-glucopyranoside, 18(C-3).

20 Yield: 15 %. Detailed characterization data have been previously reported by Nishimura, S. I.; Matsuoka, K.; Furuike, T.; Ishii, S.; Kurita, K. *Macromolecules* 1991, 24, 4236.

n-Undecenyl 2-Acetamido-2-deoxy- β -D-glucopyranoside, 18(C-9).

25 Yield: 16 %. mp: 175-177°C. $[\alpha]_D^{20} = -7.0^\circ$ (c 0.23, MeOH). ^1H NMR ($\text{CDCl}_3 + 5\%$ CD_3OD), δ_{ppm} : 1.32 (m, 9H), 1.54 (m, 2H), 2.01 (m and s, 5H), 3.32 (m, 1H), 3.47 (m, 2H), 3.54 (m, 2H), 3.84 (m, 3H), 4.47 (d, 1H, $J_{1,2} = 8.0$ Hz, *H-1*), 4.96 (m, 2H, $\text{CH}_2=$), 5.81 (m, 1H, CH=). MS/FAB, m/z : 373 (M^+).



Scheme 10. Synthesis of Nonsulfated Alkene-Derivatized Glycomonomers

5

Example 16

Synthesis of nonsulfated acrylic glycomonomers

Acrylic monomer **19** was directly synthesized from *N*-acetyl-n-Glucosamine as depicted in Scheme 11. Treatment of this carbohydrate with 2-hydroxyethyl acrylate in the presence of phosphomolybdic acid as catalyst and 1-chloro-2,4-dinitrobenzene as polymerization inhibitor yielded a crude α,β -anomeric mixture (compounds **19** and **20**, respectively) in an overall yield close to 50%. Column chromatography allowed both anomers to be separated with a ratio (α/β) of 3.5/1. They were characterized by ^1H and ^{13}C NMR, as well as by mass spectrometry. A mixture of *N*-acetyl-D-glucosamine (10 g, 45 mmol), 2-hydroxyethyl acrylate (52 g, 450 mmol), chlorobenzene (10 g, 90 mmol), phosphomolybdic acid (0.82 g, 0.45 mmol) and 1-chloro-2,4-dinitrochlorobenzene (1g, 4.5 mmol) was heated to 110 °C. The reaction was monitored by TLC; after 5 h, the reaction mixture was cooled and then neutralized with a saturated NaHCO_3 aqueous solution. The crude product obtained was purified by column chromatography with a chloroform/methanol (96/4) mixture whereby the α -anomer (Scheme 11, compound **19**) was recovered as a viscous oil and the β -anomer (Scheme 11, compound **20**) was isolated as a white powder.

25

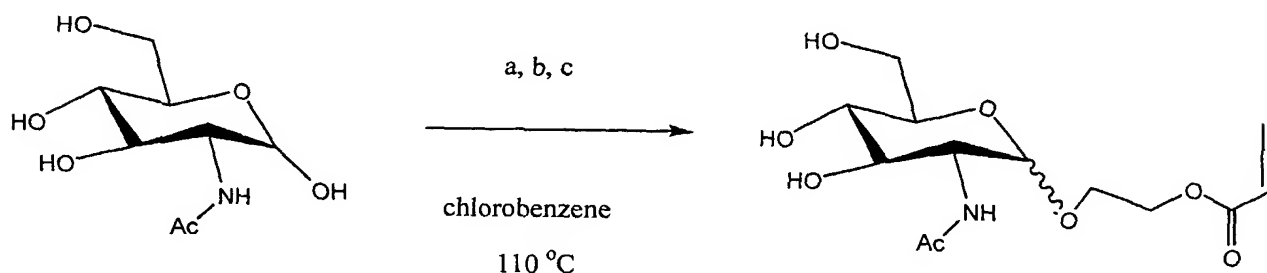
2S-(4,5-dihydroxy-6-hydroxymethyl-3-methylcarboxamidotetrahydro-2H-2-pyranlyoxy)ethyl acrylate, **19**

Yield: 37 %. $[\alpha]_D^{20} = 32.5^\circ$ (c 2, CHCl_3). ^1H NMR (CDCl_3), δ_{ppm} : 1.88 (s, 3H, NHCOCH_3), 3.38-3.66 (m, 7H), 3.81 (m, 1H), 4.17 (m, 3H), 4.70 (d, 1H, $J_{1,2} = 3.5$ Hz, $H-1$), 5.79 (d, 1H, $J = 10.5$ Hz, $\text{CH}_2=$), 6.05 (dd, 1H, $J = 10.5$ and 17.2 Hz, $\text{CH}=$), 6.29 (d, 1H, $J = 17.2$ Hz, $\text{CH}_2=$). ^{13}C NMR (CDCl_3), δ_{ppm} : 22.5, 53.3, 61.2, 63.1, 65.7, 68.6, 70.3, 71.9, 97.5, 127.7, 131.6,

5 167.0, 172.0. MS/FAB, m/z : 320 ($\text{M}^+ + \text{H}^+$).

2R-(4,5-dihydroxy-6-hydroxymethyl-3-methylcarboxamidotetrahydro-2H-2-pyraniloxy)ethyl acrylate, 20.

Yield: 11 %. mp: 138-140 $^\circ\text{C}$. $[\alpha]_D^{20} = +24.5^\circ$ (c 2, CHCl_3). ^1H NMR (CDCl_3), δ_{ppm} : 1.89 (s, 3H, NHCOCH_3), 3.28-3.48 (m, 5H), 3.35 (m, 1H), 3.44 (m, 1H), 3.64-3.78 (m, 3H), 3.91 (m, 1H), 4.17 (m, 1H), 4.28 (m, 1H), 4.43 (d, 1H, $J_{1,2} = 7.9$ Hz, $H-1$), 5.80 (d, 1H, $J = 10.4$ Hz, $\text{CH}_2=$), 6.05 (dd, 1H, $J = 10.4$ and 17.5 Hz, $\text{CH}=$), 6.36 (d, 1H, $J = 17.5$ Hz, $\text{CH}_2=$). ^{13}C NMR (CDCl_3), δ_{ppm} : 22.7, 56.2, 61.5, 63.1, 66.8, 70.6, 74.7, 75.8, 100.3, 127.8, 131.5, 166.2, 172.6. MS/FAB, m/z : 320 ($\text{M}^+ + \text{H}^+$).



N-acetyl-D-glucosamine

19 (α -anomer), 20 (β -anomer)

a, 2-hydroxyethyl acrylate; b, phosphomolybdic acid; c, 1-chloro-2,4-dinitrobenzene

Scheme 11. Synthesis of Nonsulfated and Acrylic Glycomonomers

20

Example 17*Preparation of sulfated glycomonomers*

Chemoselective sulfation of all hydroxyl groups on α -anomers, namely 17(C-3), 17(C-9), and 19, was achieved by treating these monosaccharides with $\text{SO}_3\text{-NMe}_3$ complex at 60°C . As expected, a relative downfield shift in the ^1H NMR spectra of the sulfated glycomonomers (compounds 21(C-3), 21(C-9), and 22) was observed when compared with their nonsulfated homologues. Under argon atmosphere, the appropriate amount of sulfur trioxide-trimethylamine ($\text{SO}_3\text{-NMe}_3$) complex (4 eq. for each hydroxyl group) was added to a nonsulfated glycomonomer in DMF, and the mixture was stirred at 60°C for 12 h. The reaction medium was then cooled to 0°C , and a saturated NaHCO_3 aqueous solution was added. The crude mixture was stirred for 1 h and concentrated to a smaller volume that was passed through a diethylaminoethyl (DEAE)-sephacel anion-exchange resin column. It was first eluted with a 10 mM sodium phosphate buffer (pH ~ 7.0), thereby removing the unreacted nonsulfated compound. The sulfated homologue was then eluted with a 1M NaCl buffer (pH ~ 7.0), and recovered as a mixture of its trisodium with a NaCl excess. The latter eluate was concentrated, redissolved in a minimum amount of water and passed through a Trisacryl (Gf05 M grade, Sigma-Aldrich) size-exclusion resin column for isolation of the sulfated compound free of NaCl (Scheme 12). Appropriate fractions were pooled and freeze-dried to provide the pure sulfated glycomonomer in 30-35% yield.

n-Pentenyl 2-Acetamido-2-deoxy-3,4,6-trisulfoxy- α -D-glucopyranoside, trisodium salt, 21(C-3)

$[\alpha]_{\text{D}}^{23} = +27.8^\circ$ (c 1.2, H_2O). ^1H NMR (D_2O), δ_{ppm} : 1.73 (m, 2H), 2.00 (s, 3H, NHCOCH_3), 2.17 (m, 2H), 3.51 (m, 1H), 3.76 (m, 1H), 3.90 (m, 1H), 4.23 (m, 2H), 4.25 (m, 1H), 4.59 (m, 2H), 4.90 (d, 1H, $J = 3.6$ Hz, $H-1$), 5.05 (m, 2H, $\text{CH}_2=$), 5.90 (m, 1H, $\text{CH}=\text{}$). C NMR (D_2O), δ_{ppm} : 24.8, 30.4, 32.4, 55.3, 70.1, 70.4, 71.5, 77.2, 79.4, 99.1, 117.5, 147.7, 177.1. MS/FAB, m/z : 595 ($\text{M}^+ + 3\text{Na}^+ - 3\text{H}^+$), 572 ($\text{M}^+ + 2\text{Na}^+ - 2\text{H}^+$).

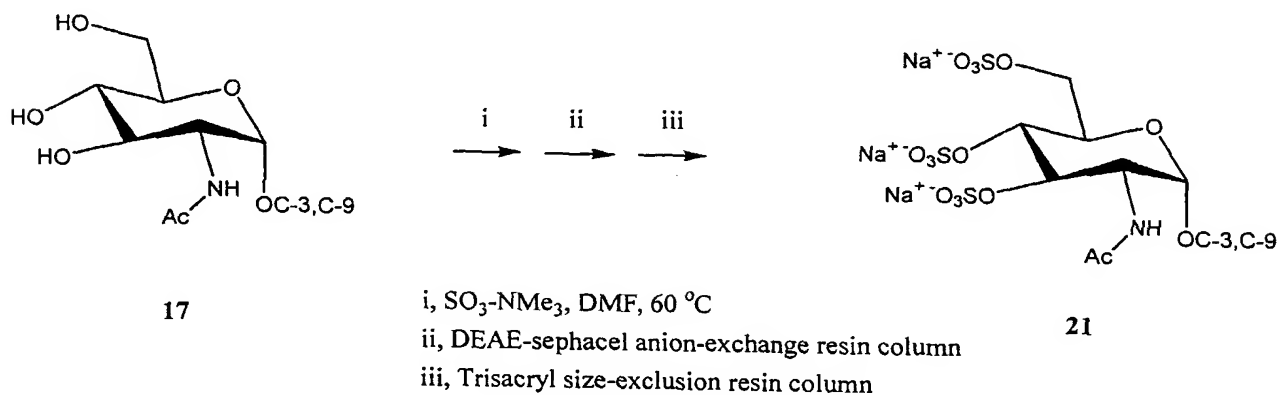
n-Undecenyl 2-Acetamido-2-deoxy-3,4,6-trisulfoxy- α -D-glucopyranoside, trisodium salt, 21(C-9). $[\alpha]_{\text{D}}^{23} = +32.0^\circ$ (c 0.2, H_2O). ^1H NMR (D_2O), δ_{ppm} : 1.35 (m, 12H), 1.61 (m, 2H), 2.05 (m, 5H), 3.50 (m, 1H), 3.73 (m, 1H), 3.91 (m, 1H), 4.19 (m, 2H), 4.28 (m, 1H), 4.58 (m, 2H),

4.91 (d, 1H, $J = 3.7$ Hz, $H-1$), 5.04 (m, 2H, $CH_2=$), 5.91 (m, 1H, $CH=$). ^{13}C NMR (D_2O), δ_{ppm} : 24.8, 35.8, 55.3, 70.0, 71.1, 71.2, 79.4, 99.0, 116.7, 143.2, 177.1. MS/FAB, m/z : 679 ($M^+ + 3Na^+ - 3H^+$).

5 2S-(4,5-disufoxy-6-sulfoxymethyl-3-methylcarboxamidotetrahydro-2H-2-pyranyloxy)ethylacrylate, trisodium salt, 22

$[\alpha]_D^{23} = -39.0^\circ$ (c 0.8, H_2O). 1H NMR (D_2O), δ_{ppm} : 1.92 (s, 3H, CH_3), 3.34-3.69 (m, 7H), 3.89 (m, 1H), 4.23 (m, 3H), 4.80 (d, 1H, $J = 3.5$ Hz, $H-1$), 5.82 (d, 1H, $J = 10.3$ Hz, $CH_2=$), 6.07 (dd, 1H, $J = 10.3$ and 17.4 Hz, $CH=$), 6.38 (d, 1H, $J = 17.4$ Hz, $CH_2=$).

10



Scheme 12. Synthesis of Sulfated Alkene-Derivatized Glycomonomers

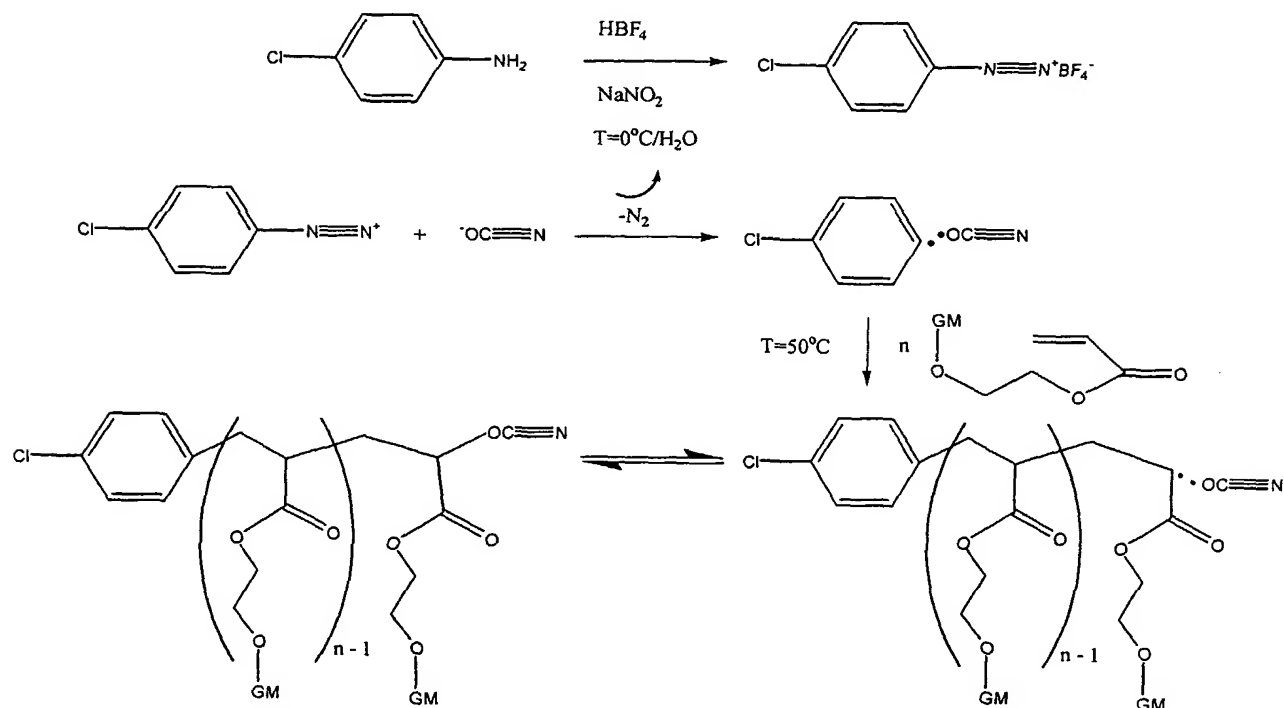
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Example 18

Statistical copolymerization of alkene-derivatized glycomonomers and acrylamide initiated by $C_6H_4N \equiv N^+BF_4^-/NaOCN$

- 20 In a three-neck flask, 6.03×10^{-5} mol (0.008 g) of *p*-chloroaniline was reacted with 9.04×10^{-5} mol. of HBf_4 (actually 0.017 g of 48 wt % aqueous solution), at 0 °C, in 2 mL of water and under argon atmosphere. The diazonium salt $C_6H_4N \equiv N^+BF_4^-$ was then generated by adding 7.2×10^{-5} mol. (0.005 g) of sodium nitrite ($NaNO_2$) to the reaction medium. After 30 minutes, a degassed mixture of 6.03×10^{-4} mol. (0.225 g) of glycomonomer 17(C-9), 2.41×10^{-3} mol. (0.171 g) of acrylamide, and 6.03×10^{-5} mol. (0.004 g) of sodium cyanate ($NaOCN$), dissolved in 1 mL of water/tetrahydrofuran (1/1), was introduced into the flask containing the diazonium salt. The polymerization medium was then heated to 50 °C. The statistical
- 25

copolymers formed after 1.5 and 16 h of reaction were isolated by precipitation in a 10-fold excess of cold methanol, dried and weighed so as to determine the conversion.



GM = nonsulfated or sulfated *N*-acetyl-D-glucosamine residue

5

Scheme 13. Cyanoxyl-Mediated Free-Radical Polymerization of Acrylic Glycomonomers

Table 6. Statistical Free-Radical Copolymerization of AM with Alkene-Derivatized Unprotected Glycomonomers Using $\text{ClC}_6\text{H}_4\text{N}\equiv\text{N}^+\text{BF}_4^-/\text{NaOCN}$ as Initiating System^a

glycomonomer (GM)	monomer ratio GM/AM (mol)	time (h)	yield ^b (%)	polymer composition ^c GM/AM (mol)	Mono-saccharide content (wt%)	M_n^d (g/mol)	M_w/M_n SEC
1 (C-3)	1/4	1.5	10	1/7	38	24,100	1.46
1 (C-3)	1/4	16	30	1/5	50	43,000	1.47
1 (C-3)	1/20	1.5	23	1/90	04	94,000	1.17
1 (C-3)	1/20	16	30	1/70	05	112,100	1.2
1 (C-9)	1/4	1.5	15	1/16	25	43,400	1.25
1 (C-9)	1/4	16	20	1/6	46	99,300	1.45
1 (C-9)	1/20	1.5	21	1/166	03	25,800	1.14
1 (C-9)	1/20	16	29	1/100	05	28,200	1.24
5 (C-3)	1/4	1.5	21	1/10	44	16,100	1.13
5 (C-3)	1/4	16	35	1/6	55	57,300	1.37
5 (C-3)	1/20	1.5	35	1/93	07	25,400	1.10
5 (C-3)	1/20	16	51	1/58	11	47,200	1.29
5 (C-9)	1/4	16	26	1/10	45	57,200	1.20
5 (C-9)	1/20	16	11	1/42	17	16,300	1.17

^a $T=50\text{ }^\circ\text{C}$, $[\text{M}]_0 = [\text{GM}]_0 + [\text{AM}]_0 = 1\text{ mol/L}$, $[\text{I}]_0 = [\text{ClC}_6\text{H}_4\text{N}\equiv\text{N}^+\text{BF}_4^-]_0 = [\text{NaOCN}]_0 = 0.02\text{ mol/L}$. ^b Total conversion of comonomers as determined by gravimetry. ^c Molar ratio of monosaccharide to acrylamide monomeric units in the resulting copolymer as determined by $^1\text{H NMR}$. ^d M_n obtained from SEC/RI/LLS.

10 Example 19

Homopolymerization of acrylic glycomonomers initiated by $\text{ClC}_6\text{H}_4\text{N}\equiv\text{N}^+\text{BF}_4^-/\text{NaOCN}$.

The general mechanism of this reaction is depicted in Scheme 13, and a typical polymerization is described hereafter. In a three-neck flask, $2.45 \times 10^{-5}\text{ mol.}$ (0.003 g) of *p*-chloroaniline was reacted with $3.67 \times 10^{-5}\text{ mol.}$ of HBF_4 (actually 0.007 g of 48 wt % aqueous solution), at $0\text{ }^\circ\text{C}$, in 2 mL of water and under argon atmosphere. The diazonium salt $\text{ClC}_6\text{H}_4\text{N}\equiv\text{N}^+\text{BF}_4^-$ was generated by adding $2.93 \times 10^{-5}\text{ mol.}$ (0.002 g) of NaNO_2 to the reaction medium. After 30 minutes, a degassed mixture of $1.22 \times 10^{-3}\text{ mol.}$ (0.39 g) of glycomonomer 19 and $2.45 \times 10^{-5}\text{ mol.}$ (0.002 g) of NaOCN , dissolved in 0.5 mL of water, was introduced into the flask containing the arenediazonium salt. The polymerization medium was then heated to $50\text{ }^\circ\text{C}$ for 4 h. The resulting glycopolymer was isolated by precipitation in a 10-fold excess of cold methanol and dried to yield a white cotton wool-like material.

Table 7. Free-Radical Homopolymerization of Acrylic Unprotected Glycomonomers and Statistical Copolymerization with AM Using $\text{ClC}_6\text{H}_4\text{N}\equiv\text{N}^+\text{BF}_4^-/\text{NaOCN}$ as Initiating System

glycomonomer (GM)	monomer ratio GM/AM (mol)	time (h)	yield ^c (%)	polymer composition ^f GM/AM (mol)	monosaccharide content (wt%)	M_n^g (g/mol)	M_w/M_n SEC
3 ^a	1/0	4	25	1/0	100	15,400	1.26
3 ^b	1/0	1.5	40	1/0	100	77,100	1.45
3 ^b	1/0	4	70	1/0	100	127,000	1.56
3 ^b	1/1	1.5	88	1/1.5	75	47,400	1.57
3 ^b	1/4	1.5	33	1/7	40	30,600	1.35
3 ^b	1/4	4	93	1/6	43	51,700	1.55
6 ^c	1/0	4	35	1/0	100	9,900	1.13
6 ^d	1/0	4	25	1/0	100	20,800	1.49
6 ^c	1/1	1.5	20	1/4	66	48,000	1.45
6 ^c	1/1	4	25	1/2	80	59,500	1.47
6 ^c	1/4	1.5	95	1/9	42	115,600	1.57

5 ^a $T=50^\circ\text{C}$, $[\text{M}]_0 = [\text{GM}]_0 + [\text{AM}]_0 = 1 \text{ mol/L}$, $[\text{I}]_0 = [\text{ClC}_6\text{H}_4\text{N}\equiv\text{N}^+\text{BF}_4^-]_0 = [\text{NaOCN}]_0 = 0.1 \text{ mol/L}$. ^b $T=50^\circ\text{C}$, $[\text{M}]_0 = 1 \text{ mol/L}$, $[\text{I}]_0 = 0.02 \text{ mol/L}$. ^c $T=50^\circ\text{C}$, $[\text{M}]_0 = 0.5 \text{ mol/L}$, $[\text{I}]_0 = 0.05 \text{ mol/L}$. ^d $T=50^\circ\text{C}$, $[\text{M}]_0 = 0.05 \text{ mol/L}$, $[\text{I}]_0 = 0.01 \text{ mol/L}$. ^e Total conversion of comonomers as determined by gravimetry. ^f Molar ratio of monosaccharide to acrylamide monomeric units in the resulting copolymer as determined by ^1H NMR. ^g M_n obtained from SEC/RI/LLS.

10 This invention has been illustrated using specific examples of linking groups, saccharides, second units, and reagents. However, as will be apparent to those skilled in the art, other equivalent components and reagents can be substituted for those specifically mentioned herein. Such equivalents are included within the scope of the following claims.

CLAIMS

1. A glycopolymer composition comprising glycopolymer molecules having:

a polymer backbone;

a first pendent unit connected to said polymer backbone comprising:

a linking group;

a saccharide moiety connected to said linking group;

an optional second pendent unit connected to said polymer backbone;

a phenyl ring at a first end of said polymer backbone; and

a cyanoxyl group at a second end of said polymer backbone.

2. The glycopolymer composition of claim 1 wherein said linking group comprises about 3 to about 9 carbon atoms.

3. The glycopolymer composition of claim 1 having a polydispersity index between about 1.1 and about 1.5.

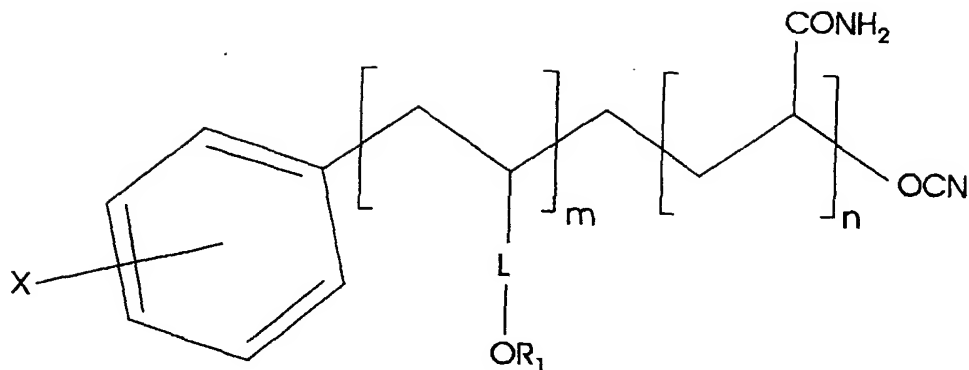
4. The glycopolymer composition of claim 1 wherein said polymer backbone is branched.

5. The glycopolymer composition of claim 1 wherein said saccharide moieties are selected from the group consisting of monosaccharides, disaccharides, trisaccharides and oligosaccharides.

6. The glycopolymer composition of claim 1 wherein said saccharide moieties comprise *N*-acetyl-D-glucosamine.

7. The glycopolymer composition of claim 1 wherein said saccharide moieties comprise
- and/or, -N-acetyl-D-glucosamine-(1 \rightarrow 4)--D-glucuronic acid.
- 5 8. The glycopolymer composition of claim 1 wherein said saccharide moieties comprise
pyranosides.
9. The glycopolymer composition of claim 1 wherein said saccharide moieties comprise
lactose.
- 10 10. The glycopolymer composition of claim 1 wherein said saccharide moieties are fully
or partially sulfated.
11. The glycopolymer composition of claim 1 wherein said first pendent units are situated
in *trans*-configuration along said polymer backbone.
- 15 12. The glycopolymer composition of claim 1 wherein said said first pendent units are
situated in *cis*-configuration along said polymer backbone.
- 20 13. The glycopolymer composition of claim 1 wherein said second unit is produced by
copolymerization with a monomer selected from the group consisting of acrylamide,
acrylate, or alkenyl compounds.
14. The glycopolymer composition of claim 13 wherein said monomer is acrylamide.
- 25 15. The glycopolymer composition of claim 13 wherein said monomer is an alkenyl
compound.
16. The glycopolymer composition of claim 1 wherein said molecules comprise between
about 2 and about 1000 said first pendent units.
- 30 17. The glycopolymer composition of claim 1 wherein the total number of said first
pendent units and second pendent units is no more than about 200.

18. The glycopolymer composition of claim 1 wherein the sum of said first pendent units and said second pendent units is no more than about 1000.
- 5 19. The glycopolymer composition of claim 1 wherein said molecules comprise up to about 1000 of said second units.
20. The glycopolymer composition of claim 1 wherein said first pendent units and said second pendent units are interspersed with each other in random order.
- 10 21. The glycopolymer composition of claim 1 wherein said phenyl ring is chlorophenyl.
22. The glycopolymer composition of claim 1 wherein said ring comprises a *para* substituent
- 15 23. The glycopolymer composition of claim 22 wherein said substituent is selected from the group consisting of hydrogen, optionally substituted hydrocarbyl, alcohol, amine, polyamine, sulfate, phosphate, halogen selected from the group consisting of chlorine, bromine and iodine, salts of the foregoing.
- 20 24. The glycopolymer composition of claim 1 bound to cytokine or growth factor molecules.
- 25 25. The glycopolymer composition of claim 1 wherein said molecules comprise hydrophilic moieties capable of rendering said composition water-soluble.
26. The glycopolymer composition of claim 1 wherein said molecules have the formula:



wherein X = H, Cl, NO₂ or OCH₃; L is a linker selected from the group of $-(CH_2)_p-$ with p between 3 and about 9, or $-COO(CH_2)_2-$; OR₁ is selected from the group consisting of saccharide moieties; m is between 2 and about 1000; and n is between 0 and about 1000, wherein individual portions of the molecule designated by m and n may be in any order.

27. A method of making a cyanoxyl-glycopolymer comprising comprising:
 - a) providing a cyanoxyl radical of an arenediazonium salt;
 - b) providing glycomonomers comprising terminal vinyl groups;
 - c) polymerizing said glycomonomers in the presence of said cyanoxyl radicals.
28. The method of claim 27 also providing providing acrylamide molecules comprising terminal vinyl groups.
29. The method of claim 27 wherein said glycomonomers are fully or partially sulfated.
30. The method of claim 27 wherein said glycomonomers are made by attaching at least one saccharide residue to an alkenyl, acrylate or acylamide linkage.

31. The method of claim 27 wherein said cyanoxyl radical is made by contacting an arenediazonium salt with a cyanate anion.
32. The method of claim 27 performed at a temperature between about 50 and about 70
5 degrees C.
33. The method of claim 28 comprising providing said glycomonomers and acrylamide
10 molecules at a ratio of about 1:4 glycomonomer:acrylamide.

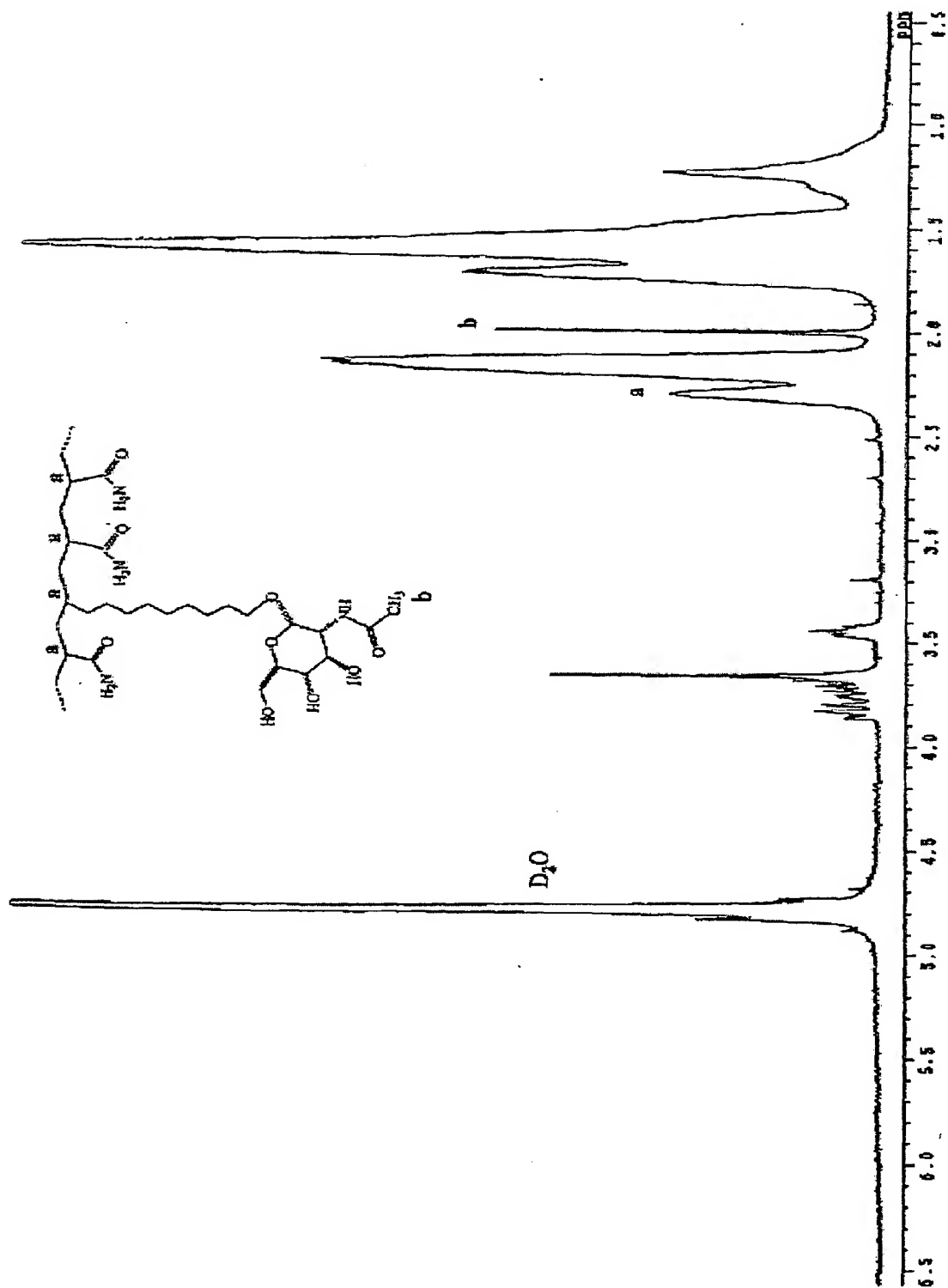


FIG. 1

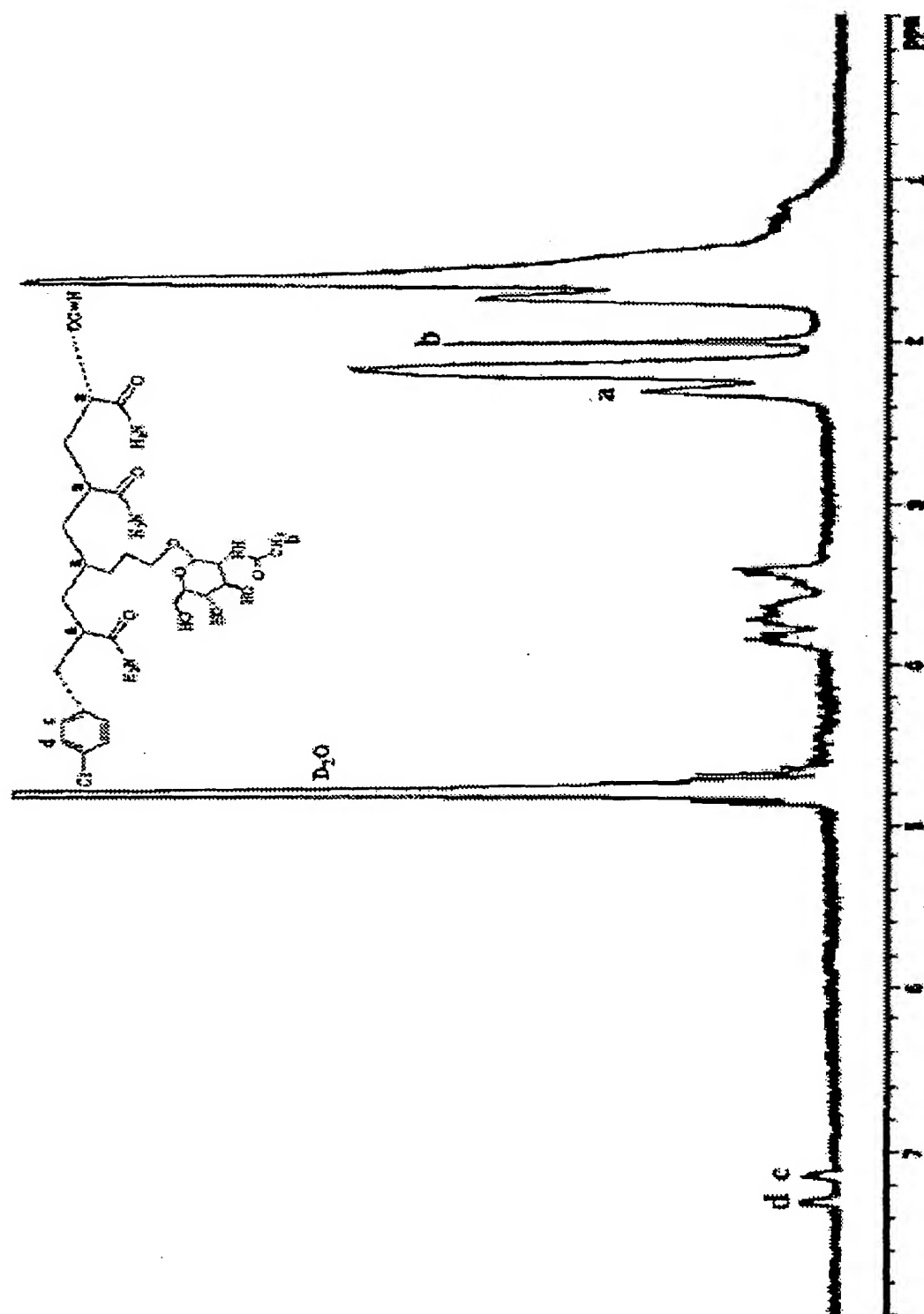


FIG. 2

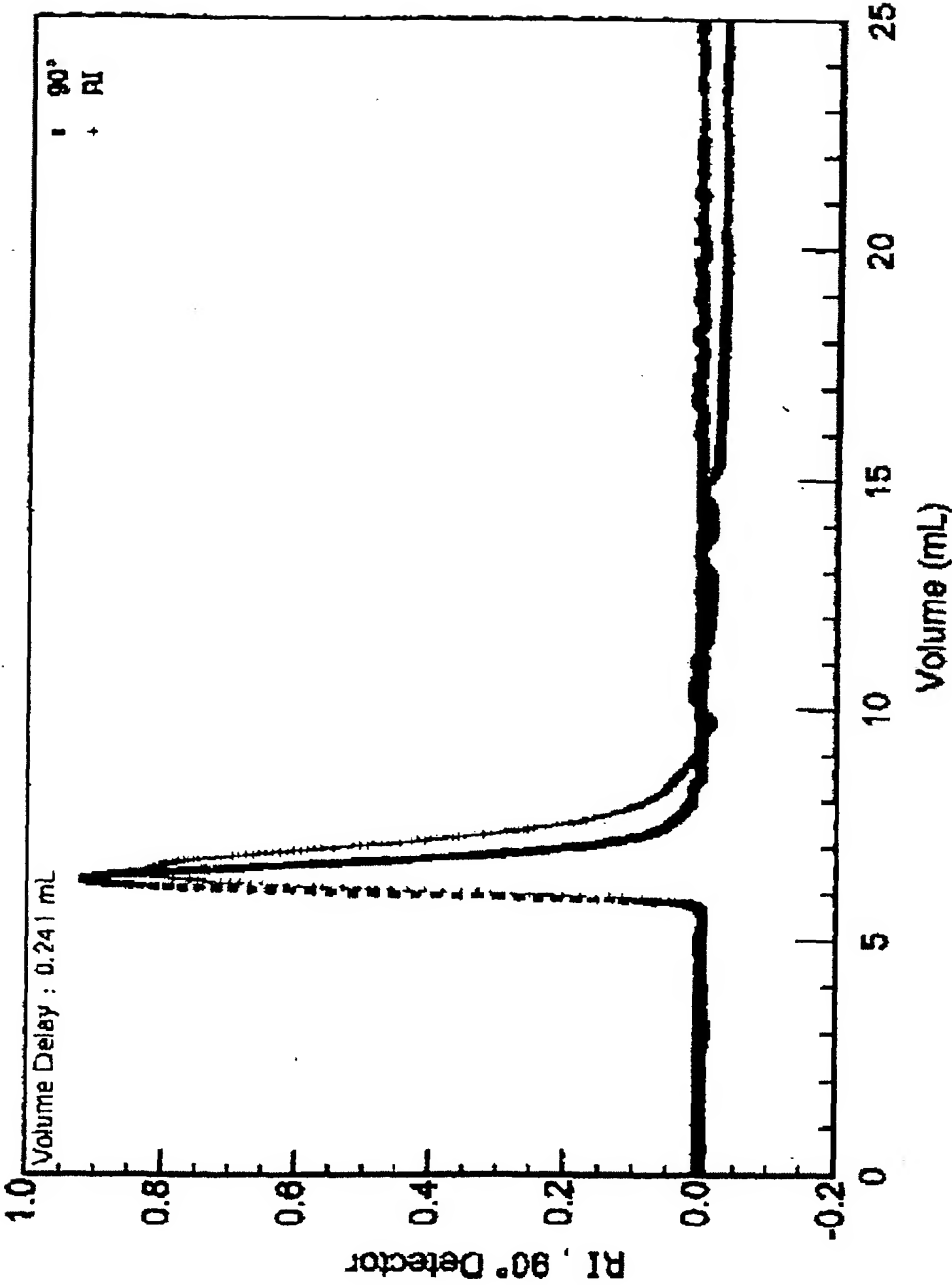


FIG. 3